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[Continued on next page]

(54) Title: G-PROTEIN COUPLED RECEPTOR NUCLEIC ACIDS, POLYPEPTIDES, ANTIBODIES AND USES THEREOF

GGCCGCCTTT GCAAGGTTGC TGGACAGATG GAACTGGAAG GGCAGCCGTC
TGCCGCCAC GAACACCTTC TCAAGCACCT TGAGTGACCA CGGCTTGCAA
GCTGTGGCT GCGCCCGGCA GTCCCGGGCT CTGAGGCAG GCGCTCGACT
TAAGCGTTGC ATCCTGTATC CTGGAGACCC TCTGAGCTCT CACCTGTCTAC
TTCTGCCGCT GCTTCTGCAC AGAGCCCGGG CGAGGACCCC TCCAGG

ATGCAGGTCC CGAACAGCAC CGGCCCGGAC AACCGCAGCG TGCAGATGCT
GCGGAACCCG GCGATCGCGG TGGCCCTGCG CGTGGTGTAC TCGCTGGTGG
CGCGGGTCAG CATCCCGGGC AACCTCTTCT CTCTGTGGGT GCTGTGCGCG
CGCATGGGGC CCAGATCCCC GTCCGGTCATC TTCAATGATCA ACCTGAGCGT
CACGGACCTG ATGCTGGCCA GCGTGTGTCC TTTCACAAATC TACTACCATT
GCAACCGCCA CCACTGGGTA TTCGGGGTGC TGCTTTGCAA CGTGGTGACC
GTGGCCTTTT ACGCAAAATG GTATTCCAGC ATCCTCACC A TGACCTGTAT
CAGCGTGGAG CGCTTCTTGG GGGTCTCTGA CCCGCTCAGC TCCAAGCGCT
GGCGCCGCGG TCGTTACGCG GTGGCCGCGT GTGCAGGGAC CTGGCTGCTG
CTCTGACCG CCCTGTCCCC GCTGGCGCGC ACCGATCTCA CCTACCCGGT
GCACGCCCTG GGCATCATCA CCTGCTTCGA CGTCTCAAG TGGACGATGC
TCCCCAGCGT GGCCATGTGG GCCGTGTCTC TCTTACCAT CTTCATCCTG
CTGTTCCTCA TCCCGTTCTGT GATCACCGTG GCTTGTATCA CGGCCACCAT
CCTCAAGCTG TTGCGCACGG AGGAGGCGCA CGGCCGGGAG CAGCGGAGGC
GCGCGGTGGG CCTGGCCGCG GTGGTCTTGC TGGCCCTTGT CACCTGTCTC
GCCCCCAACA ACTTCGTGCT CCTGGCGCAC ATCGTGAGCC GCCTGTCTTA
CGGCAAGAGC TACTACCAG TGTACAAGCT CACGCTGTGT CTCAGCTGCC
TCAACAATAG TCTGGACCCG TTTGTTTAT ACTTTGCGTC CCGGGAATTC
CAGCTGCGCC TGCGGGAATA TTTGGGCTGC CGCCGGGTGC CCAGAGACAC
CCTGGACACG CGCCGCGAGA GCCTCTTCTC CGCCAGGACC ACGTCCGTGC
GCTCCGAGGC CGGTGCGCAC CCTGAAGGGA TGGAGGGAGC CACGAGGCC
GGCTTCCAGA GGCAGGAGAG TGTGTTT

TCCCTGTGTA CATCGTCCCT TAGTTGTGGT TCTGGCCTTC TCCATCTCTC
TCCAGGGGTT CTGGTCTCCG TAGCCCGGTG CACGCCGAAA TTTCTGTCTA
TTTCACTCAG GGGCACTGTG GTTGTGTGG TTGGAATTCT TCTTTCCAGG
GAGCGCTGG GGTCTCTGCA AGTCAGCTAC TCTCCGTGCC CACTTCCCTT
CACACACACA CCCCCCTCGT GCCGAATTCT T

(57) Abstract: The invention provides isolated HGPRBMY1 and HGPRBMY2 nucleic acid molecules and polypeptide molecules. The invention also provides antisense nucleic acid molecules, expression vectors containing the nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid molecule of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and antibodies. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

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**G-PROTEIN COUPLED RECEPTOR NUCLEIC ACIDS,
POLYPEPTIDES, ANTIBODIES AND USES THEREOF**

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This application claims benefit to provisional application U.S. Serial No. 60/270,793, filed February 23, 2001; to provisional application U.S. Serial No. 60/270,792, filed February 23, 2001; and to provisional application U.S. Serial No. 60/296,427, filed June 6, 2001. The teachings of the referenced applications are incorporated herein by reference in their entirety.

10

1. INTRODUCTION

15

Many transmembrane proteins are receptors that bind a ligand and transduce an intracellular signal, leading to a variety of cellular responses. The identification and characterization of such a receptor enables one to identify both the ligands which bind to the receptor and the intracellular molecules and signal transduction pathways associated with the receptor, permitting one to identify or design modulators of receptor activity, *e.g.*, receptor agonists or antagonists and modulators of signal transduction.

20

The present invention relates to the discovery and characterization of nucleic acid molecules that encode a G-protein coupled receptor (GPCR), a receptor that participates in signal transduction in eukaryotic cells. More specifically, the present invention relates to a novel GPCR that is particularly expressed in bone marrow and spleen tissue, referred to herein as HGPRBMY1. The invention features GPCR nucleic acid molecules, host cell expression systems, GPCRs, fusion polypeptides, peptides, antibodies to the receptor, transgenic animals that express a GPCR transgene, or recombinant knock-out animals that do not express the GPCR, antagonists and agonists of the receptor, and other compounds that modulate GPCR gene expression or GPCR activity that can be used for diagnosis, drug screening, clinical trial monitoring, and/or as pharmaceutical compositions the treatment of immune related diseases and disorders, particularly proliferative immune and autoimmune disorders, specifically p27 and/or I κ B defects.

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The present invention relates to the discovery and characterization of nucleic acid molecules that encode a G-protein coupled receptor (GPCR), a receptor that participates in signal transduction in eukaryotic cells. More specifically, the present invention relates

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to a novel GPCR that is particularly expressed in heart and brain tissue, referred to herein
5 as HGPRBMY2. The invention encompasses GPCR nucleic acid molecules, host cell
expression systems, GPCR polypeptides, fusion polypeptides, peptides, antibodies to the
receptor, transgenic animals that express a GPCR transgene, or recombinant knock-out
animals that do not express the GPCR, antagonists and agonists of the receptor, and other
10 compounds that modulate GPCR gene expression or GPCR activity that can be used for
diagnosis, drug screening, clinical trial monitoring, and/or as pharmaceutical
compositions the treatment of cardiovascular and/or neural diseases and disorders.

2. BACKGROUND OF THE INVENTION

15 G-protein coupled receptors (GPCRs) belong to one of the largest receptor
superfamilies known. These receptors are biologically important and malfunction of these
receptors results in diseases such as Alzheimer's, Parkinson, diabetes, dwarfism, color
blindness, retinal pigmentosa and asthma. GPCRs are also important signaling molecules
in subjects with depression, schizophrenia, sleeplessness, hypertension, anxiety, stress,
20 renal failure and in several other cardiovascular, metabolic, neuro, oncology and immune
disorders (Horn and Vriend, J. Mol. Med. 76:464-468, 1998). They have also been shown
to play a role in HIV infection (Feng et al., (1996) Science 272:872-877).

GPCRs are integral membrane proteins characterized by the presence of seven
25 hydrophobic transmembrane domains which span the plasma membrane and form a
bundle of antiparallel alpha helices. The transmembrane domains account for structural
and functional features of the receptor. In most cases, the bundle of helices forms a
binding pocket; however, when the binding site must accommodate more bulky
molecules, the extracellular N-terminal segment or one or more of the three extracellular
30 loops participate in binding and in subsequent induction of conformational change in
intracellular portions of the receptor. The activated receptor, in turn, interacts with an
intracellular heterotrimeric G-protein complex which mediates further intracellular
signaling activities, generally interaction with guanine nucleotide binding (G) proteins
and the production of second messengers such as cyclic AMP (cAMP), phospholipase C,
35 inositol triphosphate or ion channel proteins (Baldwin, J. M. (1994) Curr. Opin. Cell
Biol. 6:180-190). The activity of the receptors are then modulated by modification, such
as phosphorylation, or by binding to a regulatory molecule, such as by the negative

regulatory molecule arrestin, or by internalization wherein the receptor is degraded in a
5 lysosome (see generally Hu, L.A., *et al.*, (2000) J. Biol. Chem.. 275:38659-38666).

The amino-terminus of the GPCR is extracellular, of variable length and often
glycosylated, while the carboxy-terminus is cytoplasmic. Extracellular loops of the GPCR
alternate with intracellular loops and link the transmembrane domains. The most
10 conserved domains of GPCRs are the transmembrane domains and the first two
cytoplasmic loops. GPCRs range in size from under 400 to over 1000 amino acids
(Coughlin, S. R. (1994) Curr. Opin. Cell Biol. 6:191-197).

GPCRs respond to a diverse array of ligands including lipid analogs, amino acids
and their derivatives, peptides, cytokines, and specialized stimuli such as light, taste, and
15 odor. GPCRs function in physiological processes including vision (the rhodopsins), smell
(the olfactory receptors), neurotransmission (muscarinic acetylcholine, dopamine, and
adrenergic receptors), and hormonal response (luteinizing hormone and
thyroid-stimulating hormone receptors).

GPCR mutations, both of the loss-of-function and of the activating variety, have
20 been associated with numerous human diseases (Coughlin, *supra*). For instance, retinitis
pigmentosa may arise from either loss-of-function or activating mutations in the
rhodopsin gene. Somatic activating mutations in the thyrotropin receptor cause
hyperfunctioning thyroid adenomas (Parma, J. *et al.* (1993) Nature 365:649-651). Parma
25 *et al.* suggest that certain G-protein-coupled receptors susceptible to constitutive
activation may behave as proto-oncogenes.

Characterization of the HGPRBMY1 polypeptide of the present invention led to
the determination that it is involved in the modulation of the cyclin p27 protein, in
addition to, the apoptosis regulatory protein I κ B, either directly or indirectly. The present
30 invention represents the first association between HGPRBMY1 to cell cycle and
apoptosis regulation.

Critical transitions through the cell cycle are highly regulated by distinct protein
kinase complexes, each composed of a cyclin regulatory and a cyclin-dependent kinase
(cdk) catalytic subunit (for review see Draetta, 1994). These proteins regulate the cell's
35 progression through the stages of the cell cycle and are in turn regulated by numerous
proteins, including p53, p21, p16, p27, and cdc25. Downstream targets of cyclin-cdk
complexes include pRb and E2F. The cell cycle often is dysregulated in neoplasia due to

alterations either in oncogenes that indirectly affect the cell cycle or in tumor suppressor
5 genes or oncogenes that directly impact cell cycle regulation, such as pRb, p53, p16,
cyclin D1, or mdm-2 (for review see Lee and Yang, 2001, Schafer, 1998).

P27, also known as CDKN1B (cyclin-dependent kinase inhibitor 1B) or KIP1,
shares a limited similarity with the CDK inhibitor CDKN1A/p21. The encoded protein
binds to and prevents the activation of cyclinE-CDK2 or cyclinD-CDK4 complexes.
10 Therefore it mainly blocks the cell cycle progression at the G1- and S-phases (for review
see Desdouets and Brechot, 2000).

Reduction in levels of p27 and increased expression of cyclin E also occur and
carry a poor prognostic significance in many common forms of cancer. The inhibition of
15 protein activities leading to an upregulation of p27 might therefore be a possibility to
decrease the progression of cancer and increase patient survival rates (for review see
Sgambato, 2000).

Recently, Medema et al. (2000) demonstrated that p27 is a major transcriptional
target of forkhead transcription factors FKHRL1, AFX, or FKHR. Overexpression of
20 these proteins causes growth suppression in a variety of cell lines, including a Ras-
transformed cell line and a cell line lacking the tumor suppressor PTEN integrating
signals from PI3K/PKB signaling and RAS/RAL signaling to regulate transcription of
p27(KIP1). Expression of AFX blocked cell cycle progression at phase G1, independent
25 of functional retinoblastoma protein but dependent on the cell cycle inhibitor p27 (KIP1).
This is further supported by the fact that AFX activity inhibits p27 -/- knockout mouse
cells significantly less than their p27 +/- counterparts.

The connection between the PTEN pathway and the activation of p27 via
forkhead-like transcription factors implies that genes whose inhibition leads to p27
30 upregulation might be involved in this pathway. Therefore the identification of genes
whose knockout leads to an upregulation of p27 might be useful drug targets, as
inhibition of such genes should result in the upregulation of p27 and therefore be useful
for the treatment and/or amelioration of cancer and increase a cancer patients prolonged
outlook and survival.

35 The fate of a cell in multicellular organisms often requires choosing between life
and death. This process of cell suicide, known as programmed cell death or apoptosis,
occurs during a number of events in an organisms life cycle, such as for example, in

development of an embryo, during the course of an immunological response, or in the
5 demise of cancerous cells after drug treatment, among others. The final outcome of cell
survival versus apoptosis is dependent on the balance of two counteracting events, the
onset and speed of caspase cascade activation (essentially a protease chain reaction), and
the delivery of antiapoptotic factors which block the caspase activity (Aggarwal B.B.
10 Biochem. Pharmacol. 60, 1033-1039, (2000); Thornberry, N. A. and Lazebnik, Y.
Science 281, 1312-1316, (1998)).

The production of antiapoptotic proteins is controlled by the transcriptional factor
complex NF- κ B. For example, exposure of cells to the protein tumor necrosis factor
(TNF) can signal both cell death and survival, an event playing a major role in the
15 regulation of immunological and inflammatory responses (Ghosh, S., May, M. J., Kopp,
E. B. Annu. Rev. Immunol. 16, 225-260, (1998); Silverman, N. and Maniatis, T., Genes
& Dev. 15, 2321-2342, (2001); Baud, V. and Karin, M., Trends Cell Biol. 11, 372-377,
(2001)). The anti-apoptotic activity of NF- κ B is also crucial to oncogenesis and to
chemo- and radio-resistance in cancer (Baldwin, A.S., J. Clin. Inves. 107, 241-246,
20 (2001)).

Nuclear Factor- κ B (NF- κ B), is composed of dimeric complexes of p50 (NF- κ B1)
or p52 (NF- κ B2) usually associated with members of the Rel family (p65, c-Rel, Rel B)
which have potent transactivation domains. Different combinations of NF- κ B/Rel
25 proteins bind distinct κ B sites to regulate the transcription of different genes. Early work
involving NF- κ B suggested its expression was limited to specific cell types, particularly
in stimulating the transcription of genes encoding kappa immunoglobulins in B
lymphocytes. However, it has been discovered that NF- κ B is, in fact, present and
inducible in many, if not all, cell types and that it acts as an intracellular messenger
30 capable of playing a broad role in gene regulation as a mediator of inducible signal
transduction. Specifically, it has been demonstrated that NF- κ B plays a central role in
regulation of intercellular signals in many cell types. For example, NF- κ B has been
shown to positively regulate the human beta-interferon (beta-IFN) gene in many, if not
all, cell types. Moreover, NF- κ B has also been shown to serve the important function of
35 acting as an intracellular transducer of external influences.

The transcription factor NF- κ B is sequestered in an inactive form in the cytoplasm
as a complex with its inhibitor, I κ B, the most prominent member of this class being I κ B α .

A number of factors are known to serve the role of stimulators of NF- κ B activity, such
5 as, for example, TNF. After TNF exposure, the inhibitor is phosphorylated and
proteolytically removed, releasing NF- κ B into the nucleus and allowing its transcriptional
activity. Numerous genes are upregulated by this transcription factor, among them I κ Ba.
The newly synthesized I κ Ba protein inhibits NF- κ B, effectively shutting down further
transcriptional activation of its downstream effectors. However, as mentioned above, the
10 I κ Ba protein may only inhibit NF- κ B in the absence of I κ Ba stimuli, such as TNF
stimulation, for example. Other agents that are known to stimulate NF- κ B release, and
thus NF- κ B activity, are bacterial lipopolysaccharide, extracellular polypeptides,
chemical agents, such as phorbol esters, which stimulate intracellular phosphokinases,
15 inflammatory cytokines, IL-1, oxidative and fluid mechanical stresses, and Ionizing
Radiation (Basu, S., Rosenzweig, K. R., Youmell, M., Price, B. D, Biochem, Biophys,
Res, Commun., 247(1):79-83, (1998)). Therefore, as a general rule, the stronger the
insulting stimulus, the stronger the resulting NF- κ B activation, and the higher the level
of I κ Ba transcription. As a consequence, measuring the level of I κ Ba RNA can be used
20 as a marker for antiapoptotic events, and indirectly, for the onset and strength of pro-
apoptotic events.

The upregulation of I κ Ba due to the downregulation of HGPRBMY1 places this
GPCR protein into a signalling pathway potentially involved in apoptotic events. This
25 gives the opportunity to regulate downstream events via the activity of the protein
HGPRBMY1 with antisense polynucleotides, polypeptides or low molecular chemicals
with the potential of achieving a therapeutic effect in cancer, and autoimmune diseases.
In addition to cancer and immunological disorders, NF- κ B has significant roles in other
diseases (Baldwin, A. S., J. Clin Invest. 107, :3-6 (2001)). NF- κ B is a key factor in the
30 pathophysiology of ischemia-reperfusion injury and heart failure (Valen, G., Yan. ZQ,
Hansson, GK, J. Am. Coll. Cardiol. 38, 307-14 (2001)). Furthermore, NF- κ B has been
found to be activated in experimental renal disease (Guijarro C, Egido J., Kidney Int. 59,
415-425 (2001)). As HGPRBMY1 is highly expressed in bone marrow and spleen and
there is the potential of an involvement in immune diseases.

35 The discovery of a new human G-protein coupled receptor as described herein,
and the nucleic acids encoding it satisfies a need in the art by providing new
compositions which are useful in the diagnosis, prevention and treatment of immune

disorders, and particularly those G-protein coupled receptors that modulate the p27
5 and/or NFkB pathways.

3. SUMMARY OF THE INVENTION

HGPRBMY1 is a putative G-protein coupled receptor (GPCR) that is expressed
in tissues, in particular immune system tissues such as bone marrow, spleen and thymus.
10 More specifically, HGPRBMY1 comprises the amino acid sequences depicted in Figure
2 which is encoded by the nucleic acid sequence depicted in Figure 1.

HGPRBMY2 is predicted to be a G-protein coupled receptor (GPCR) that is
expressed in heart and brain tissue. More specifically, HGPRBMY2 comprises the amino
15 acid sequences depicted in Figure 7 which is encoded by the nucleic acid sequence
depicted in Figure 6. The clone encoding the HGPRBMY2 polypeptide was deposited
with the ATCC as ATCC Deposit Number XXXXXX on XXXXXX.

As HGPRBMY1 and HGPRBMY2 have homology to GPCRs, they are likely
seven transmembrane proteins located at the membrane of a cell. Signal transduction
20 from GPCRs is triggered by the binding of agonists or antagonists to the receptor.
Secondary regulation of the receptor may occur through post-stimulatory modification
of the polypeptide (*e.g.*, phosphorylation) and/or by binding to a secondary regulatory
molecule, particularly on a cytoplasmic domain of the receptor (*e.g.*, arrestin).

HGPRBMY1 mRNA has been detected in the bone marrow, spleen and thymus.
25 Thus, neutralization of HGPRBMY1 agonists or antagonists, removal of HGPRBMY1
agonists or antagonists, or interference with binding to HGPRBMY1 may result in
improvement or prevention of immune related disease.

HGPRBMY2 mRNA has been detected in the heart, and various tissues of the
30 brain. Thus, neutralization of HGPRBMY2 agonists or antagonists, removal of
HGPRBMY2 agonists or antagonists, or interference with binding to HGPRBMY2 may
result in improvement or prevention of cardiovascular and/or neurological diseases.

The invention features the use of HGPRBMY1 nucleic acid molecules,
HGPRBMY1 polypeptides and peptides, fusion polypeptides or fusion peptides (*e.g.*,
35 fusions to heterologous sequences), as well as antibodies to the HGPRBMY1 (which can,
for example, act as HGPRBMY1 agonists or antagonists), antagonists that inhibit
receptor activity or expression, or agonists that activate receptor activity or increase its

expression in the diagnosis and treatment of immune system or immune response
5 diseases and/or disorders including, but not limited to immune system diseases or
disorders in animals, including humans, particularly proliferative immune disorders, and
autoimmune disorders.

The invention features the use of HGPRBMY2 nucleic acid molecules,
HGPRBMY2 polypeptides and peptides, fusion polypeptides or fusion peptides (*e.g.*,
10 fusions to heterologous sequences), as well as antibodies to the HGPRBMY2 (which can,
for example, act as HGPRBMY2 agonists or antagonists), antagonists that inhibit
receptor activity or expression, or agonists that activate receptor activity or increase its
expression in the diagnosis and treatment of the cardiovascular system diseases or
15 disorders, in addition to neural disorders, in animals, including humans.

The diagnosis of an HGPRBMY1 abnormality in a patient, or an abnormality in
the HGPRBMY1 signal transduction pathway, will assist in devising a proper treatment
or therapeutic regimen for immune disorders. In addition, HGPRBMY1 nucleic acid
molecules and HGPRBMY1 polypeptides are useful for the identification of compounds
20 effective in the treatment of immune disorders regulated by the HGPRBMY1, particularly
proliferative immune disorders, and autoimmune disorders.

The diagnosis of an HGPRBMY2 abnormality in a patient, or an abnormality in
the HGPRBMY2 signal transduction pathway, will assist in devising a proper treatment
25 or therapeutic regimen for heart failure. In addition, HGPRBMY2 nucleic acid molecules
and HGPRBMY2 polypeptides are useful for the identification of compounds effective
in the treatment of cardiovascular and/or neural disorders regulated by the HGPRBMY2.

In particular, the invention described in the subsections below features
HGPRBMY1, polypeptides or peptides corresponding to functional domains of the
30 HGPRBMY1 (*e.g.*, extracellular domain (ECD), transmembrane domain (TM) or
cytoplasmic domain (CD)), mutated, truncated or deleted HGPRBMY1 (*e.g.*, an
HGPRBMY1 with one or more functional domains or portions thereof deleted, such as
 Δ TM and/or Δ CD), HGPRBMY1 fusion polypeptides (*e.g.*, an HGPRBMY1 or a
functional domain of HGPRBMY1, such as the ECD, fused to an unrelated polypeptide
35 or peptide such as an immunoglobulin constant region, *i.e.*, Ig-Fc), nucleic acid sequences
encoding such products, and host cell expression systems that can produce such
HGPRBMY1 products.

The invention also features antibodies and anti-idiotypic antibodies (including
5 Fab fragments), antagonists and agonists of the HGPRBMY1, as well as compounds or
nucleic acid constructs that inhibit expression of the HGPRBMY1 gene (transcription
factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence
replacement constructs), or promote expression of HGPRBMY1 (*e.g.*, expression
10 constructs in which HGPRBMY1 coding sequences are operatively associated with
expression control elements such as promoters, promoter/enhancers, etc.). The invention
also relates to host cells and animals genetically engineered to express the human
HGPRBMY1 (or mutants thereof) or to inhibit or "knock-out" expression of the animal's
endogenous HGPRBMY1.

15 The HGPRBMY1 polypeptides or peptides, HGPRBMY1 fusion polypeptides,
HGPRBMY1 nucleic acid sequences, antibodies, antagonists and agonists can be useful
for the detection of mutant HGPRBMY1 or inappropriately expressed HGPRBMY1 for
the diagnosis of immune disorders. The HGPRBMY1 polypeptides or peptides,
HGPRBMY1 fusion polypeptides, HGPRBMY1 nucleic acid sequences, host cell
20 expression systems, antibodies, antagonists, agonists and genetically engineered cells and
animals can be used for screening for drugs effective in the treatment of such immune
disorders. The use of engineered host cells and/or animals may offer an advantage in that
such systems allow not only for the identification of compounds that bind to the ECD of
the HGPRBMY1, but can also identify compounds that affect the signal transduced by
25 the activated HGPRBMY1.

The HGPRBMY2 polypeptides or peptides, HGPRBMY2 fusion polypeptides,
HGPRBMY2 nucleic acid sequences, antibodies, antagonists and agonists can be useful
for the detection of mutant HGPRBMY2 or inappropriately expressed HGPRBMY2 for
30 the diagnosis of heart disease or neural disorders. The HGPRBMY2 polypeptides or
peptides, HGPRBMY2 fusion polypeptides, HGPRBMY2 nucleic acid sequences, host
cell expression systems, antibodies, antagonists, agonists and genetically engineered cells
and animals can be used for screening for drugs effective in the treatment of such heart
disease or immune disorders. The use of engineered host cells and/or animals may offer
35 an advantage in that such systems allow not only for the identification of compounds that
bind to the ECD of the HGPRBMY2, but can also identify compounds that affect the
signal transduced by the activated HGPRBMY2.

The HGPRBMY1 polypeptide products (especially soluble derivatives such as
5 peptides corresponding to the HGPRBMY1 ECD, or soluble polypeptides lacking one
or more TM domains ("ΔTM")), fusion polypeptides (especially HGPRBMY1-Ig fusion
polypeptides, *i.e.*, fusions of the HGPRBMY1 or a domain of the HGPRBMY1, *e.g.*,
ECD, ΔTM or CD to a heterologous sequence such as IgFc), antibodies and anti-idiotypic
10 antibodies (including Fab fragments), antagonists or agonists (including compounds that
modulate signal transduction which may act on downstream targets in the HGPRBMY1
signal transduction pathway) can be used for therapy of such diseases. For example, the
administration of an effective amount of a pharmaceutical composition comprising
soluble HGPRBMY1 ECD, ΔTM HGPRBMY1 or an ECD-IgFc fusion polypeptide or
15 an anti-idiotypic antibody (or its Fab) that mimics the HGPRBMY1 ECD would
modulate endogenous HGPRBMY1 agonists or antagonists, and prevent or reduce
binding and receptor activation, leading to prevention of immune disorders.

For example, the administration of an effective amount of a pharmaceutical
composition comprising a fusion polypeptide or an anti-idiotypic antibody, or fragment
20 thereof, that mimics HGPRBMY1 would modulate endogenous HGPRBMY1 binding
to signaling partners, leading to treatment of immune disorders, particularly proliferative
immune disorders, and autoimmune disorders.

For example, the administration of an effective amount of a pharmaceutical
composition comprising soluble HGPRBMY2 ECD, ΔTM HGPRBMY2 or an ECD-IgFc
25 fusion polypeptide or an anti-idiotypic antibody (or its Fab) that mimics the HGPRBMY2
ECD would modulate endogenous HGPRBMY2 agonists or antagonists, and prevent or
reduce binding and receptor activation, leading to prevention of heart failure.

Nucleic acid constructs encoding such HGPRBMY1 products can be used to
30 genetically engineer host cells to express such HGPRBMY1 products *in vivo*; these
genetically engineered cells, when placed in the body, deliver a continuous supply of
HGPRBMY1 polypeptides or peptides, that modulate HGPRBMY1 activity. Nucleic acid
constructs encoding functional HGPRBMY1, mutant HGPRBMY1, or antisense and
ribozyme molecules can be used in gene therapy approaches for the modulation of
35 HGPRBMY1 activity in the treatment of immune disorders, particularly proliferative
immune disorders, and autoimmune disorders.

Nucleic acid constructs encoding such HGPRBMY2 products can be used to
5 genetically engineer host cells to express such HGPRBMY2 products *in vivo*; these
genetically engineered cells deliver a continuous supply of soluble HGPRBMY2 peptide,
ECD or Δ TM or HGPRBMY2 fusion polypeptide that will modulate activation of
HGPRBMY2 by agonists or antagonists. Nucleic acid constructs encoding functional
10 HGPRBMY2, mutant HGPRBMY2, as well as antisense and ribozyme molecules can be
used in "gene therapy" approaches for the modulation of HGPRBMY2 expression and/or
activity in the treatment of heart disease or neural disorders.

The invention also features HGPRBMY1 pharmaceutical formulations and
methods for treating immune disorders, particularly proliferative immune disorders, and
15 autoimmune disorders.

Thus, the invention also encompasses HGPRBMY2 pharmaceutical formulations
and methods for treating heart or neural diseases.

The invention further relates to a method of identifying a compound that
modulates the biological activity of HGPRBMY1 or HGPRBMY2, comprising the steps
20 of, (a) combining a candidate modulator compound with HGPRBMY1 or HGPRBMY2
having the sequence set forth in one or more of SEQ ID NO:2; and measuring an effect
of the candidate modulator compound on the activity of HGPRBMY1 or HGPRBMY2.

The invention further relates to a method of identifying a compound that
modulates the biological activity of a GPCR, comprising the steps of, (a) combining a
25 candidate modulator compound with a host cell expressing HGPRBMY1 or
HGPRBMY2 having the sequence as set forth in SEQ ID NO:2; and , (b) measuring an
effect of the candidate modulator compound on the activity of the expressed
HGPRBMY1 or HGPRBMY2.

30 The invention further relates to a method of identifying a compound that
modulates the biological activity of HGPRBMY1 or HGPRBMY2, comprising the steps
of, (a) combining a candidate modulator compound with a host cell containing a vector
described herein, wherein HGPRBMY1 or HGPRBMY2 is expressed by the cell; and,
35 (b) measuring an effect of the candidate modulator compound on the activity of the
expressed HGPRBMY1 or HGPRBMY2.

The invention further relates to a method of screening for a compound that is

capable of modulating the biological activity of HGPRBMY1 or HGPRBMY2,
5 comprising the steps of: (a) providing a host cell described herein; (b) determining the
biological activity of HGPRBMY1 or HGPRBMY2 in the absence of a modulator
compound; (c) contacting the cell with the modulator compound; and (d) determining
the biological activity of HGPRBMY1 or HGPRBMY2 in the presence of the modulator
10 compound; wherein a difference between the activity of HGPRBMY1 or HGPRBMY2
in the presence of the modulator compound and in the absence of the modulator
compound indicates a modulating effect of the compound.

The invention further relates to a recombinant host cell comprising a vector
comprising all or a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 ,
15 NFAT/CRE, and/or NFAT G alpha 15 wherein said host cell exhibits low levels of
HGPRBMY1 or HGPRBMY2 expression. Such host cells are particularly useful in
methods of screening for agonists of the HGPRBMY1 or HGPRBMY2 polypeptide.

The invention further relates to a recombinant host cell comprising a vector
20 comprising all or a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 ,
NFAT/CRE, and/or NFAT G alpha 15 wherein said host cell exhibits intermediate levels
of HGPRBMY1 or HGPRBMY2 expression. Such host cells are particularly useful in
methods of screening for modulators of the HGPRBMY1 or HGPRBMY2 polypeptide.

The invention further relates to a recombinant host cell comprising a vector
25 comprising all or a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 ,
NFAT/CRE, and/or NFAT G alpha 15 wherein said host cell exhibits high levels of
HGPRBMY1 or HGPRBMY2 expression. Such host cells are particularly useful in
methods of screening for antagonists of the HGPRBMY1 or HGPRBMY2 polypeptide.

The invention further relates to a method of screening for candidate compounds
30 capable of modulating activity of a G-protein coupled receptor-encoding polypeptide,
comprising the steps of contacting a test compound with a cell or tissue expressing all or
a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 , NFAT/CRE, and/or
NFAT G alpha 15 wherein said cell or tissue exhibits low, intermediate, or high
35 HGPRBMY1 or HGPRBMY2 expression levels, and selecting as candidate modulating
compounds those test compounds that modulate activity of the the HGPRBMY1 or
HGPRBMY2 polypeptide.

5 The invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a proliferative disorder.

10 More preferably, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an antagonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a proliferative disorder.

15 More preferably, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an antagonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a disorder related to aberrant apoptosis regulation.

20 Alternatively, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an agonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a proliferative disorder.

25 More preferably, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an agonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a disorder related to aberrant apoptosis regulation.

30 The invention further relates to peptides that bind to the HGPRBMY1 or HGPRBMY2 polypeptide. More preferred are peptides that modulate the activity of HGPRBMY1 or HGPRBMY2 activity.

35 The invention further relates to a method for identifying compounds that regulate immune-related disorders, comprising the step of contacting a test compound with a cell which expresses a nucleic acid of SEQ ID NO:1, and determining whether the test compound modulates HGPRBMY1 activity.

The invention further relates to a method for identifying compounds
5 that regulate immune-related disorders comprising the step of contacting a test
compound with a nucleic acid of SEQ ID NO:1; and determining whether the test
compound interacts with the nucleic acid of SEQ ID NO:1.

The invention further relates to a method for identifying compounds
that regulate immune-related disorders, comprising the step of contacting a test
10 compound with a cell or cell lysate containing a reporter gene operatively associated
with a HGPRBMY1 regulatory element; and detecting expression of the reporter gene
product.

The invention further relates to a method for identifying compounds
that regulate immune-related disorders comprising the step of contacting a test
15 compound with a cell or cell lysate containing HGPRBMY1 transcripts; and detecting
the translation of the HGPRBMY1 transcript.

The invention further relates to a method for modulating
immune-related disorders in a subject, comprising administering to the subject a
therapeutically effective amount of a HGPRBMY1 polypeptide.

20 The invention further relates to a method for modulating immune-related
disorders in a subject, comprising administering to the subject a therapeutically
effective amount of a HGPRBMY1 polypeptide wherein the HGPRBMY1
polypeptide is HGPRBMY1 or a functionally equivalent derivative thereof, preferably
wherein the subject is a human.

25 The invention further relates to a method for modulating immune-related
disorders in a subject, comprising administering to the subject a therapeutically
effective amount of a HGPRBMY1 polypeptide wherein the HGPRBMY1
polypeptide is HGPRBMY1 or a functionally equivalent derivative thereof, preferably
wherein the subject is a human, wherein the HGPRBMY1 polypeptide is contained in
30 a pharmaceutical composition.

The invention further relates to a method for the treatment of immune-related
disorders, comprising modulating the activity of a HGPRBMY1 polypeptide.

The invention further relates to a method for the treatment of immune-related
disorders, comprising modulating the activity of a HGPRBMY1 polypeptide, wherein
35 the HGPRBMY1 polypeptide is HGPRBMY1 or a functionally equivalent derivative
thereof.

The invention further relates to a method for the treatment of
5 immune-related disorders, comprising modulating the activity of a HGPRBMY1
polypeptide, wherein the HGPRBMY1 polypeptide is HGPRBMY1 or a functionally
equivalent derivative thereof, wherein the method comprises administering an
effective amount of a compound that agonizes or antagonizes the activity of the
HGPRBMY1 polypeptide.

10 The invention further relates to a method for the treatment of immune-related
disorders, comprising administering an effective amount of a compound that
decreases expression of a HGPRBMY1 gene.

The invention further relates to a method for the treatment of
immune-related disorders, comprising administering an effective amount of a
15 compound that decreases expression of a HGPRBMY1 gene, wherein the compound
is an oligonucleotide encoding an antisense or ribozyme molecule that targets
HGPRBMY1 transcripts and inhibits translation.

The invention further relates to a method for the treatment of
immune-related disorders, comprising administering an effective amount of a
20 compound that decreases expression of a HGPRBMY1 gene, wherein the compound
is an oligonucleotide that forms a triple helix with the promoter of the HGPRBMY1
gene and inhibits transcription.

The invention further relates to a method for the treatment of
immune-related disorders, comprising administering an effective amount of a
25 compound that increases expression of a HGPRBMY1 gene.

The invention further relates to a pharmaceutical formulation for the
treatment of immune-related disorders, comprising a compound that activates or
inhibits HGPRBMY1 activity, mixed with a pharmaceutically acceptable carrier.

The invention further relates to a method for identifying compounds
30 that modulate the activity of a G-protein coupled receptor comprising the step of
(a) contacting a test compound to a cell that expresses a HGPRBMY1 gene and the
G-protein coupled receptor, and measuring activity; (b) contacting a test
compound to a cell that expresses a HGPRBMY1 gene but does not express the
G-protein coupled receptor, and measuring activity; and (c) comparing activity
35 obtained in (b) with the activity obtained in (a); such that if the level obtained in (b)
differs from that obtained in (b), a compound that modulates G-protein coupled
receptor activity is identified

The invention further relates to a method for identifying compounds
5 that regulate heart-related disorders, comprising the step of contacting a test
compound with a cell which expresses a nucleic acid of SEQ ID NO:13, and
determining whether the test compound modulates HGPRBMY2 activity.

The invention further relates to a method for identifying compounds
that regulate heart-related disorders comprising the step of contacting a test compound
10 with a nucleic acid of SEQ ID NO:13; and determining whether the test compound
interacts with the nucleic acid of SEQ ID NO:13.

The invention further relates to a method for identifying compounds
that regulate heart-related disorders, comprising the step of contacting a test
compound with a cell or cell lysate containing a reporter gene operatively associated
15 with a HGPRBMY2 regulatory element; and detecting expression of the reporter gene
product.

The invention further relates to a method for identifying compounds
that regulate heart-related disorders comprising the step of contacting a test compound
with a cell or cell lysate containing HGPRBMY2 transcripts; and detecting the
20 translation of the HGPRBMY2 transcript.

The invention further relates to a method for modulating heart-related
disorders in a subject, comprising administering to the subject a therapeutically
effective amount of a HGPRBMY2 polypeptide.

The invention further relates to a method for modulating heart-related
25 disorders in a subject, comprising administering to the subject a therapeutically
effective amount of a HGPRBMY2 polypeptide wherein the HGPRBMY2
polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof, preferably
wherein the subject is a human.

The invention further relates to a method for modulating heart-related
30 disorders in a subject, comprising administering to the subject a therapeutically
effective amount of a HGPRBMY2 polypeptide wherein the HGPRBMY2
polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof, preferably
wherein the subject is a human, wherein the HGPRBMY2 polypeptide is contained in
a pharmaceutical composition.

35 The invention further relates to a method for the treatment of heart-related
disorders, comprising modulating the activity of a HGPRBMY2 polypeptide.

The invention further relates to a method for the treatment of heart-related disorders, comprising modulating the activity of a HGPRBMY2 polypeptide, wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof.

The invention further relates to a method for the treatment of heart-related disorders, comprising modulating the activity of a HGPRBMY2 polypeptide, wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof, wherein the method comprises administering an effective amount of a compound that agonizes or antagonizes the activity of the HGPRBMY2 polypeptide.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene, wherein the compound is an oligonucleotide encoding an antisense or ribozyme molecule that targets HGPRBMY2 transcripts and inhibits translation.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene, wherein the compound is an oligonucleotide that forms a triple helix with the promoter of the HGPRBMY2 gene and inhibits transcription.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that increases expression of a HGPRBMY2 gene.

The invention further relates to a pharmaceutical formulation for the treatment of heart-related disorders, comprising a compound that activates or inhibits HGPRBMY2 activity, mixed with a pharmaceutically acceptable carrier.

The invention further relates to a method for identifying compounds that modulate the activity of a G-protein coupled receptor comprising the step of (a) contacting a test compound to a cell that expresses a HGPRBMY2 gene and the G-protein coupled receptor, and measuring activity; (b) contacting a test compound to a cell that expresses a HGPRBMY2 gene but does not express the

- G-protein coupled receptor, and measuring activity; and (c) comparing activity
5 obtained in (b) with the activity obtained in (a); such that if the level obtained in (b) differs from that obtained in (b), a compound that modulates G-protein coupled receptor activity is identified

3.1 DEFINITIONS

- 10 The term "derivative" as used herein refers to a polypeptide that comprises an amino acid sequence of a GPCR polypeptide or peptide as described herein that has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a GPCR polypeptide or peptide that has
15 been modified, *i.e.*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, a GPCR polypeptide or peptide may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other polypeptide, etc. A derivative of a GPCR polypeptide
20 or peptide may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a GPCR polypeptide or peptide may contain one or more non-classical amino acids. A polypeptide derivative possesses a similar or identical function as a GPCR polypeptide or peptide
25 described herein.

- An "isolated" or "purified" polypeptide or polypeptide complex of the invention is substantially free of cellular material or other contaminating polypeptides from the cell or tissue source from which the polypeptide is derived, or substantially free of chemical
30 precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a polypeptide or polypeptide complex in which the polypeptide or polypeptide complex is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a polypeptide or polypeptide complex that is substantially free of cellular material includes preparations
35 of polypeptide or polypeptide complex having less than about 30%, 20%, 10%, or 5% (by dry weight) of a heterologous polypeptide (also referred to herein as a "contaminating polypeptide"). When the polypeptide or polypeptide complex is recombinantly produced,

it is also preferably substantially free of culture medium, *i.e.*, culture medium represents
5 less than about 20%, 10%, or 5% of the volume of the polypeptide preparation. When the
polypeptide or polypeptide complex is produced by chemical synthesis, it is preferably
substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from
chemical precursors or other chemicals which are involved in the synthesis of the
10 polypeptide. Accordingly such preparations of the polypeptide or polypeptide complex
have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or
compounds other than the polypeptide or polypeptide complex of interest. In a preferred
embodiment, polypeptides or polypeptide complexes or peptides of the invention are
isolated or purified.

15 An "isolated" nucleic acid molecule is one which is separated from other nucleic
acid molecules which are present in the natural source of the nucleic acid molecule.
Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be
substantially free of other cellular material, or culture medium when produced by
recombinant techniques, or substantially free of chemical precursors or other chemicals
20 when chemically synthesized.

"Plasmids" are designated by a lower case p preceded and/or followed by capital
letters and/or numbers. The starting plasmids herein are either commercially available,
publicly available on an unrestricted basis, or can be constructed from available plasmids
25 in accord with published procedures. In addition, equivalent plasmids to those described
are known in the art and will be apparent to the ordinarily skilled artisan.

The term "fusion polypeptide" as used herein refers to a polypeptide that
comprises an amino acid sequence of a polypeptide or peptide and an amino acid
sequence of another polypeptide or peptide (*e.g.*, GPCR fused to an epitope tag such as
30 a hexa-histidine motif, or a GPCR domain fused to another GPCR domain, such as two
or more extracellular domains in tandem).

The term "GPCR antigen" refers to a GPCR polypeptide or peptide to which an
antibody or antibody fragment immunospecifically binds. A GPCR antigen also refers to
an analog or derivative of a GPCR polypeptide or peptide to which an antibody or
35 antibody fragment immunospecifically binds.

The term "antibodies or antibody fragments that immunospecifically bind to a
GPCR antigen" as used herein refers to antibodies, Fab's of antibodies, or other binding

portions of antibodies, that specifically bind to a either a native and/or denatured GPCR
5 polypeptide or a GPCR peptide and do not non-specifically bind to other polypeptides.
Antibodies, or Fab portions thereof, that immunospecifically bind to a GPCR polypeptide
or peptide may have cross-reactivity with other antigens. Preferably, antibodies or
fragments that immunospecifically bind to a GPCR polypeptide or peptide do not cross-
10 react with other antigens. Antibodies or fragments that immunospecifically bind to a
GPCR polypeptide can be identified, for example, by immunoassays or other techniques
known to those of skill in the art.

The term "patient in need thereof" refers to a human with, or at risk of, a disease
or disorder associated with the gene or gene product of the invention. Further this term
15 includes in certain embodiments immunocompromised patients. For research purposes,
an animal model, for example a mouse model or monkey model, can be utilized to
simulate such a patient in some circumstances.

To determine the percent identity of two amino acid sequences or of two nucleic
acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps
20 can be introduced in the sequence of a first amino acid or nucleic acid sequence for
optimal alignment with a second amino acid or nucleic acid sequence). The amino acid
residues or nucleic acids at corresponding amino acid positions or nucleic acid positions
are then compared. When a position in the first sequence is occupied by the same amino
acid residue or nucleic acid as the corresponding position in the second sequence, then
25 the molecules are identical at that position. The percent identity between the two
sequences is a function of the number of identical positions shared by the sequences (*i.e.*,
 $\% \text{ identity} = \text{number of identical overlapping positions} / \text{total number of positions} \times 100\%$). In one embodiment, the two sequences are the same length.

30 The determination of percent identity between two sequences can also be
accomplished using a mathematical algorithm. A preferred, non-limiting example of a
mathematical algorithm utilized for the comparison of two sequences is the algorithm of
Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in
Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an
35 algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al.,
1990, J. Mol. Biol. 215:403. BLAST nucleic acid searches can be performed with the
NBLAST nucleic acid program parameters set, *e.g.*, for score=100, wordlength=12 to

obtain nucleic acid sequences homologous to a nucleic acid molecules of the present
5 invention. BLAST polypeptide searches can be performed with the XBLAST program
parameters set, *e.g.*, to score=50, wordlength=3 to obtain amino acid sequences
homologous to a polypeptide molecule of the present invention. To obtain gapped
alignments for comparison purposes, Gapped BLAST can be utilized as described in
Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can
10 be used to perform an iterated search which detects distant relationships between
molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the
default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be
used (*e.g.*, <http://www.ncbi.nlm.nih.gov>). Another preferred, non-limiting example of a
15 mathematical algorithm utilized for the comparison of sequences is the algorithm of
Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the
ALIGN program (version 2.0) which is part of the GCG sequence alignment software
package. When utilizing the ALIGN program for comparing amino acid sequences, a
PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be
20 used.

The percent identity between two sequences can be determined using techniques
similar to those described above, with or without allowing gaps. In calculating percent
identity, typically only exact matches are counted.

25

4. DESCRIPTION OF THE FIGURES

The file of this patent contains at least one Figure executed in color. Copies of
this patent with color Figure(s) will be provided by the Patent and Trademark Office upon
30 request and payment of the necessary fee.

Figure 1: Nucleic acid sequence of the coding region of HGPRBMY1. The 5'
untranslated region is the first group of sequences, the second group of sequences is the
open reading frame of HGPRBMY1 and the third set is the 3' untranslated region.

Figure 2: Theoretical translation of the open reading frame of the cDNA of Figure
35 1, resulting in the polypeptide sequence of HGPRBMY1.

Figure 3: The shaded sequences in the polypeptide sequence in the upper half of
5 the figure reflect the transmembrane regions. The bottom of the figure depicts a
hydropathy plot of the polypeptide sequence of Figure 2.

Figure 4: Sequence alignment of HGPRBMY1 and related G-protein coupled
receptors. The GCG pileup program was used to generate the alignment. The blackened
10 areas represent identical amino acids in more than half of the listed sequences and the
grey highlighted areas represent similar amino acids.

Figure 5: Expression profile of HGPRBMY1 in various tissues as measured by
PCR. The PCR data was converted into a relative assessment of the difference in
transcript abundance amongst the tissues tested. Transcripts corresponding to the orphan
15 GPCR, HGPRBMY1, are expressed most highly in bone marrow, spleen and thymus.

Figure 6: Nucleic acid sequence of the coding region of HGPRBMY2. The 5'
untranslated region is the first group of sequences, the second group of sequences is the
open reading frame of HGPRBMY2 and the third set is the 3' untranslated region.

Figure 7: Theoretical translation of the open reading frame of the cDNA of Figure
20 6, resulting in the polypeptide sequence of HGPRBMY2.

Figure 8: The shaded sequences in the polypeptide sequence in the upper half of
the figure reflect the transmembrane regions. The bottom of the figure depicts a
Hydropathy plot of the polypeptide sequence of Figure 7.

Figure 9: Sequence alignment of HGPRBMY2 and related G-protein coupled
25 receptors. The GCG pileup program was used to generate the alignment. The blackened
areas represent identical amino acids in more than half of the listed sequences and the
grey highlighted areas represent similar amino acids.

Figure 10: Expression profile of HGPRBMY2 in various tissues as measured by
30 PCR. The PCR data was converted into a relative assessment of the difference in
transcript abundance amongst the tissues tested. Transcripts corresponding to the orphan
GPCR, HGPRBMY2, are expressed most highly in testis, heart, and thymus.

Figure 11: Untransfected Cho NFAT-CRE cell line FACS profile. Control Cho-
NFAT/CRE (Nuclear Factor Activator of Transcription (NFAT) / cAMP response
35 element (CRE)) cell lines were incubated with 10 nM PMA and 1 uM Thapsigargin / 10
uM Forskolin, respectively, in the absence of the pcDNA3.1 HygroTM / HGPRBMY2
mammalian expression vector transfection, as described herein. The stimulated cells were

sorted via FACS (Fluorescent Assisted Cell Sorter) according to their wavelength
5 emission at 518 nM (Channel R3 - Green Cells), and 447 nM (Channel R2 - Blue Cells).
As shown, the vast majority of cells emit at 518 nM, with minimal emission observed at
447 nM. The latter is expected since the NFAT/CRE response elements remain dormant
in the absence of an activated G-protein dependent signal transduction pathway (e.g.,
10 pathways mediated by Gq/11 or Gs coupled receptors). As a result, the cell permeant,
CCF2/AMTM (Aurora Biosciences; Zlokarnik, et al., 1998) substrate remains intact and
emits light at 518 nM.

Figure 12: Overexpression Of BMY2 Constitutively Couples Through The
NFAT/CRE Response Element. Cho-NFAT/CRE cell lines transfected with the
15 pcDNA3.1 HygroTM / HGPRBMY2 mammalian expression vector were incubated with
10 nM PMA and 1 μ M Thapsigargin / 10 μ M Forskolin, respectively, as described herein.
The stimulated cells were sorted via FACS according to their wavelength emission at 518
nM (Channel R3 - Green Cells), and 447 nM (Channel R2 - Blue Cells). As shown,
overexpression of HGPRBMY2 results in functional coupling and subsequent activation
20 of beta lactamase gene expression, as evidenced by the significant number of cells with
fluorescent emission at 447 nM relative to the non-transfected control Cho-NFAT/CRE
cells (shown in Figure 11).

Figure 13: HGPRBMY2 Does Not Couple Through The cAMP Response
25 Element. HEK-CRE cell lines transfected with the pcDNA3.1 HygroTM / HGPRBMY2
mammalian expression vector were incubated with 10 nM PMA and 10 μ M Forskolin,
as described herein. The stimulated cells were sorted via FACS according to their
wavelength emission at 518 nM (Channel R3 - Green Cells), and 447 nM (Channel R2
- Blue Cells). As shown, overexpression of HGPRBMY2 in te HEK-CRE cells did not
30 result in functional coupling, as evidenced by the insignificant background level of cells
with fluorescent emission at 447 nM.

Figure 14: Expressed HGPRBMY2 Localizes To The Plasma Membrane. Cho-
NFAT/CRE cell lines transfected with the pcDNA3.1 HygroTM / HGPRBMY2-FLAG
35 mammalian expression vector were subjected to immunocytochemistry using an FITC
conjugated Anti Flag monoclonal antibody, as described herein. Panel A shows the
transfected Cho-NFAT/CRE cells under visual wavelengths, and panel B shows the
fluorescent emission of the same cells at 530 nm after illumination with a laser at 447

nm. The plasma membrane localization is clearly evident in panel B, and is consistent
5 with the HGPRBMY2 polypeptide representing a member of the GPCR family.

Figure 15: Transfected Cho-NFAT/CRE cell lines With Intermediate and High
Beta Lactamase Expression Levels Useful In Screens to Identify HGPRBMY2 Agonists
and/or Antagonists. Several Cho-NFAT/CRE cell lines transfected with the pcDNA3.1
HygroTM / HGPRBMY2 mammalian expression vector were isolated via FACS that had
10 either intermediate or high beta lactamase expression levels post stimulation with 10 nM
PMA and 1 uM Thapsigargin / 10 uM Forskolin, as described herein. Panel A shows
HGPRBMY2 transfected Cho-NFAT/CRE cells prior to stimulation with 10 nM PMA
and 1 uM Thapsigargin / 10 uM Forskolin (- P/T/F). Panel B shows HGPRBMY2
15 transfected Cho-NFAT/CRE cells after stimulation with 10 nM PMA and 1 uM
Thapsigargin / 10 uM Forskolin (+ P/T/F). Panel C shows HGPRBMY2 transfected Cho-
NFAT/CRE cells after stimulation with 10 nM PMA and 1 uM Thapsigargin / 10 uM
Forskolin (+ P/T/F) that have an intermediate level of beta lactamase expression. Panel
D shows HGPRBMY2 transfected Cho-NFAT/CRE cells after stimulation with 10 nM
20 PMA and 1 uM Thapsigargin / 10 uM Forskolin (+ P/T/F) that have a high level of beta
lactamase expression.

Figure 16: Expanded Expression Profile Of The Novel Human G-Protein
Coupled Receptor, HGPRBMY2. The figure illustrates the relative expression level of
25 HGPRBMY2 amongst various mRNA tissue sources. As shown, the HGPRBMY2
polypeptide was predominately expressed in the heart, with highest expression in the left
ventricle, significantly in tissues of the posterior hypothalamus (1000-fold greater than
most other tissues), the DRG, and to a lesser extent in tissues throughout the brain in
addition to other tissues as shown. Expression data was obtained by measuring the steady
30 state HGPRBMY2 mRNA levels by quantitative PCR using the PCR primer pair
provided as SEQ ID NO:25 and 26, and Taqman probe (SEQ ID NO:27) as described
herein.

5. DETAILED DESCRIPTION OF THE INVENTION

35 HGPRBMY1 is a novel receptor expressed in bone marrow, spleen and thymus.
The present invention use of HGPRBMY1 nucleic acids, HGPRBMY1 polypeptides and
peptides, as well as antibodies to the HGPRBMY1 (which can, for example, act as

detection of mutant HGPRBMY1 or inappropriately expressed HGPRBMY1, particularly
5 for the diagnosis of immune disorders either related to HGPRBMY1 expression,
activation or down regulation, or wherein HGPRBMY1 serves as an indicator of an
immune disorder. The HGPRBMY1 polypeptides, HGPRBMY1 fusion polypeptides,
HGPRBMY1 nucleic acid sequences, host cell expression systems, antibodies,
10 antagonists, agonists and genetically engineered cells and animals can be used for
screening for drugs effective in the treatment of such immune disorders. The use of
engineered host cells and/or animals may offer an advantage in that such systems allow
not only for the identification of compounds that bind to the ECD or to the CD of the
HGPRBMY1, and/or can be used to identify compounds that modulate the signal
15 transduced by the activated HGPRBMY1.

Finally, the HGPRBMY1 polypeptide products (especially derivatives such as
peptides corresponding to a HGPRBMY1 ECD, or truncated polypeptides lacking a
hydrophobic TM domain, which are soluble under normal physiological conditions) and
fusion polypeptide products (especially HGPRBMY1-Ig fusion polypeptides, *i.e.*, fusions
20 of a domain of HGPRBMY1, *e.g.*, ECD, Δ TM or CD to a heterologous sequence such
as IgFc), antibodies (including fragments thereof), antagonists or agonists (including
compounds that modulate signal transduction which may act on downstream targets in
the HGPRBMY1 signal transduction pathway) can be used for therapy of such diseases.
25 For example, the administration of an effective amount of a pharmaceutical composition
comprising a soluble ECD, CD, Δ TM, CD-IgFc fusion, ECD-IgFc fusion polypeptide or
an antibody (or fragment thereof) that mimics the HGPRBMY1 ECD would modulate
HGPRBMY1 activity, leading to prevention or treatment of an immune disorder.

Nucleic acid constructs encoding the HGPRBMY1 products above can be used
30 to engineer host cells to express such HGPRBMY1 products *in vivo*. These implanted
cells, when implanted into a host, deliver a continuous supply of a soluble ECD or a
fusion polypeptide that modulates HGPRBMY1 activity. Nucleic acid constructs
encoding functional HGPRBMY1, mutant HGPRBMY1, as well as antisense and
ribozyme molecules can be used in gene therapy for the modulation of HGPRBMY1
35 expression and/or activity in the treatment of immune disorders. Thus, the invention
features pharmaceutical formulations and methods for treating immune disorders.

The strong homology to human G-protein coupled receptors, combined with the

HGPRBMY1 agonists or antagonists), antagonists that inhibit receptor activity or
5 expression, or agonists that activate receptor activity or increase its expression in the
diagnosis and treatment of immune disorders, including, but not limited to immune
disorders in animals, including humans. The diagnosis of abnormality associated with
HGPRBMY1 in a patient, or an abnormality in the HGPRBMY1 signal transduction
10 pathway, will assist in devising a proper treatment or therapeutic regimen. In addition,
HGPRBMY1 nucleic acids and HGPRBMY1 polypeptides are useful for the
identification of compounds effective in the treatment of immune disorders regulated by
HGPRBMY1.

The invention features HGPRBMY1 polypeptides or portions of the full length
15 polypeptide, *i.e.*, peptides, which can be designed to correspond to functional domains
of the HGPRBMY1 (*e.g.*, full length polypeptide, ECD, TM or CD), or mutated,
truncated or deleted HGPRBMY1 (*e.g.* an HGPRBMY1 with one or more functional
domains or portions thereof deleted, such as Δ TM and/or Δ CD), or HGPRBMY1 fusion
20 polypeptides (*e.g.* an HGPRBMY1 or a functional domain of HGPRBMY1, such as an
ECD fused to an unrelated polypeptide or peptide such as an immunoglobulin constant
region, *i.e.*, IgFc), nucleic acid sequences encoding such products, and host cell
expression systems that can produce such HGPRBMY1 products.

The invention also features antibodies and anti-idiotypic antibodies (including
25 antibody fragments), antagonists and agonists of the HGPRBMY1, as well as compounds
or nucleic acid constructs that inhibit expression of the HGPRBMY1 gene (transcription
factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence
replacement constructs), or promote expression of HGPRBMY1 (*e.g.*, expression
constructs in which HGPRBMY1 coding sequences are operatively associated with
30 expression control elements such as promoters, promoter/enhancers, etc.).

The invention also features host cells or animals genetically engineered to express
exogenous HGPRBMY1 (or mutants thereof), cells or animals engineered to increase
expression of the endogenous HGPRBMY1, cells or animals engineered to express a
mutated HGPRBMY1, or cells or animals engineered to inhibit expression of either an
35 animal's endogenous HGPRBMY1.

The HGPRBMY1 polypeptides, HGPRBMY1 fusion polypeptides, HGPRBMY1
nucleic acid sequences, antibodies, antagonists and agonists can be useful for the

predominate localized expression in bone marrow and spleen, in conjunction with the p27
5 and I κ B association, suggests the HGPRBMY1 polynucleotides and polypeptides may
be useful in treating, diagnosing, prognosing, and/or preventing immune diseases and/or
disorders. Representative uses are described elsewhere herein. Briefly, the strong
expression in immune tissue indicates a role in regulating the proliferation; survival;
10 differentiation; and/or activation of hematopoietic cell lineages, including blood stem
cells.

The HGPRBMY1 polypeptide may also be useful as a preventative agent for
immunological disorders including arthritis, asthma, immunodeficiency diseases such as
AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel
15 disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-
cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as
host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as
autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus,
drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.
20 The HGPRBMY1 polypeptide may be useful for modulating cytokine production, antigen
presentation, or other processes, such as for boosting immune responses, etc.

Moreover, the protein may represent a factor that influences the differentiation
or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury.
25 Thus, this gene product is thought to be useful in the expansion of stem cells and
committed progenitors of various blood lineages, and in the differentiation and/or
proliferation of various cell types. Furthermore, the protein may also be used to determine
biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or
receptors, to identify agents that modulate their interactions, in addition to its use as a
30 nutritional supplement. Protein, as well as, antibodies directed against the protein may
show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Various aspects of the invention are described in greater detail in the subsections
below.

HGPRBMY2, described for the first time herein, is a novel receptor protein
35 expressed in heart, brain tissues, testis, and thymus tissues. The invention encompasses
the use of HGPRBMY2 nucleic acids, HGPRBMY2 proteins and peptides, as well as
antibodies to the HGPRBMY2 (which can, for example, act as HGPRBMY2 agonists or

antagonists), antagonists that inhibit receptor activity or expression, or agonists that
5 activate receptor activity or increase its expression in the diagnosis and treatment of
cardiovascular disorders, including, but not limited to heart disease in animals, including
humans. The diagnosis of an HGPRBMY2 abnormality in a patient, or an abnormality
in the HGPRBMY2 signal transduction pathway, will assist in devising a proper
10 treatment or therapeutic regimen. In addition, HGPRBMY2 nucleic acids and
HGPRBMY2 proteins are useful for the identification of compounds effective in the
treatment of cardiovascular disorders regulated by the HGPRBMY2.

Expanded analysis of HGPRBMY2 expression levels by TaqMan™ quantitative
PCR (see Figure 16) confirmed that the HGPRBMY2 polypeptide is expressed at very
15 low levels in heart and testis, with relatively low-level expression in the brain sub regions
tested as shown using the SYBR green experiments (see Figure 10). HGPRBMY2 mRNA
was expression predominately in heart, with the highest concentration in the left
ventricle, and the posterior hypothalamus; significantly in the DRG and other tissues
throughout the brain, and to a lesser extent in the spinal cord in addition to other tissues
20 as shown. These data suggest that HGPRBMY2 may be useful for the treatment and/or
amelioration of metabolic disorders, mainly obesity, and for the treatment of pain
disorders.

The strong homology to human G-protein coupled receptors, combined with the
25 predominate localized expression in heart tissue suggests the HGPRBMY2
polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing,
and/or preventing cardiovascular diseases and/or disorders, which include, but are not
limited to: myocardio infarction, congestive heart failure, arrhythmias, cardiomyopathy,
atherosclerosis, arterialsclerosis, microvascular disease, embolism, thromobosis,
30 pulmonary edema, palpitation, dyspnea, angina, hypotension, syncope, heart murmer,
aberrant ECG, hypertrophic cardiomyopathy, the Marfan syndrome, sudden death,
prolonged QT syndrome, congenital defects, cardiac viral infections, valvular heart
disease, and hypertension.

Similarly, HGPRBMY2 polynucleotides and polypeptides may be useful for
35 ameliorating cardiovascular diseases and symptoms which result indirectly from various
non-cardiavascular effects, which include, but are not limited to, the following, obesity,
smoking, Down syndrome (associated with endocardial cushion defect); bony

abnormalities of the upper extremities (associated with atrial septal defect in the Holt-
5 Oram syndrome); muscular dystrophies (associated with cardiomyopathy);
hemochromatosis and glycogen storage disease (associated with myocardial infiltration
and restrictive cardiomyopathy); congenital deafness (associated with prolonged QT
interval and serious cardiac arrhythmias); Raynaud's disease (associated with primary
10 pulmonary hypertension and coronary vasospasm); connective tissue disorders, i.e., the
Marfan syndrome, Ehlers-Danlos and Hurler syndromes, and related disorders of
mucopolysaccharide metabolism (aortic dilatation, prolapsed mitral valve, a variety of
arterial abnormalities); acromegaly (hypertension, accelerated coronary atherosclerosis,
conduction defects, cardiomyopathy); hyperthyroidism (heart failure, atrial fibrillation);
15 hypothyroidism (pericardial effusion, coronary artery disease); rheumatoid arthritis
(pericarditis, aortic valve disease); scleroderma (cor pulmonale, myocardial fibrosis,
pericarditis); systemic lupus erythematosus (valvulitis, myocarditis, pericarditis);
sarcoidosis (arrhythmias, cardiomyopathy); postmenopausal effects, Chlamydial
infections, polycystic ovary disease, thyroid disease, alcoholism, diet, and exfoliative
20 dermatitis (high-output heart failure), for example.

Moreover, polynucleotides and polypeptides, including fragments and/or
antagonists thereof, have uses which include, directly or indirectly, treating, preventing,
diagnosing, and/or prognosing the following, non-limiting, cardiovascular infections:
25 blood stream invasion, bacteremia, sepsis, *Streptococcus pneumoniae* infection, group
a streptococci infection, group b streptococci infection, *Enterococcus* infection,
nonenterococcal group D streptococci infection, nonenterococcal group C streptococci
infection, nonenterococcal group G streptococci infection, *Streptococcus viridans* infection,
Staphylococcus aureus infection, coagulase-negative staphylococci infection, gram-
30 negative Bacilli infection, Enterobacteriaceae infection, *Pseudomonas* spp. Infection,
Acinobacter spp. Infection, *Flavobacterium meningosepticum* infection, *Aeromonas* spp.
Infection, *Stenotrophomonas maltophilia* infection, gram-negative coccobacilli infection,
Haemophilus influenza infection, *Branhamella catarrhalis* infection, anaerobe infection,
Bacteriodes fragilis infection, *Clostridium* infection, fungal infection, *Candida* spp.
35 Infection, non-albicans *Candida* spp. Infection, *Hansenula anomala* infection, *Malassezia*
furfur infection, nontuberculous *Mycobacteria* infection, *Mycobacterium avium* infection,
Mycobacterium chelonae infection, *Mycobacterium fortuitum* infection, spirochetal

infection, *Borrelia burgdorferi* infection, in addition to any other cardiovascular disease
5 and/or disorder (e.g., non-sepsis) implicated by the causative agents listed above or
elsewhere herein.

The strong homology to human G-protein coupled receptor proteins, combined
with the localized expression in various brain tissues suggests HGPRBMY2
10 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing,
and/or preventing neurodegenerative disease states, behavioral disorders, or inflammatory
conditions. Representative uses are described in the section 5.6c below, in the Examples,
and elsewhere herein. Briefly, the uses include, but are not limited to the detection,
treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's
15 Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral
neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia
and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia,
obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS,
psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns,
20 balance, and perception. In addition, elevated expression of this gene product in regions
of the brain indicates it plays a role in normal neural function. Potentially, this gene
product is involved in synapse formation, neurotransmission, learning, cognition,
homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also
25 be used to determine biological activity, to raise antibodies, as tissue markers, to isolate
cognate ligands or receptors, to identify agents that modulate their interactions, in
addition to its use as a nutritional supplement. Protein, as well as, antibodies directed
against the protein may show utility as a tumor marker and/or immunotherapy targets for
the above listed tissues.

30 Alternatively, the strong homology to G-protein coupled receptors, combined
with the predominate localized expression in testis tissue suggests the potential utility for
HGPRBMY2 polynucleotides and polypeptides in treating, diagnosing, prognosing,
and/or preventing testicular, in addition to reproductive disorders.

35 In preferred embodiments, HGPRBMY2 polynucleotides and polypeptides
including agonists and fragments thereof, have uses which include treating, diagnosing,
prognosing, and/or preventing the following, non-limiting, diseases or disorders of the

testis: spermatogenesis, infertility, Klinefelter's syndrome, XX male, epididymitis, genital
5 warts, germinal cell aplasia, cryptorchidism, varicocele, immotile cilia syndrome, and
viral orchitis. The HGPRBMY2 polynucleotides and polypeptides including agonists and
fragments thereof, may also have uses related to modulating testicular development,
embryogenesis, reproduction, and in ameliorating, treating, and/or preventing testicular
proliferative disorders (e.g., cancers, which include, for example, choriocarcinoma,
10 Nonseminoma, seminoma, and testicular germ cell tumors).

Likewise, the predominate localized expression in testis tissue also emphasizes
the potential utility for HGPRBMY2 polynucleotides and polypeptides in treating,
diagnosing, prognosing, and/or preventing metabolic diseases and disorders which
15 include the following, not limiting examples: premature puberty, incomplete puberty,
Kallman syndrome, Cushing's syndrome, hyperprolactinemia, hemochromatosis,
congenital adrenal hyperplasia, FSH deficiency, and granulomatous disease, for example.

This gene product may also be useful in assays designed to identify binding
20 agents, as such agents (antagonists) are useful as male contraceptive agents. The testes
are also a site of active gene expression of transcripts that is expressed, particularly at low
levels, in other tissues of the body. Therefore, this gene product may be expressed in
other specific tissues or organs where it may play related functional roles in other
processes, such as hematopoiesis, inflammation, bone formation, and kidney function,
25 to name a few possible target indications.

The strong homology to G-protein coupled receptors, combined with the localized
expression in thymus tissue suggests the HGPRBMY2 polynucleotides and polypeptides
may be useful in treating, diagnosing, prognosing, and/or preventing immune diseases
and/or disorders. The strong expression in immune tissue indicates a role in regulating
30 the proliferation; survival; differentiation; and/or activation of hematopoietic cell
lineages, including blood stem cells.

The HGPRBMY2 polypeptide may also be useful as a preventative agent for
immunological disorders including arthritis, asthma, immunodeficiency diseases such as
35 AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel
disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-
cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as

5 host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. The HGPRBMY2 polypeptide may be useful for modulating cytokine production, antigen presentation, or other processes, such as for boosting immune responses, etc.

10 Moreover, the protein may represent a factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine
15 biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The invention features HGPRBMY2 polypeptides or portions of the full length
20 polypeptide, *i.e.*, peptides, which can be designed to correspond to functional domains of the HGPRBMY2 (*e.g.*, full length protein, ECD, TM or CD), or mutated, truncated or deleted HGPRBMY2 (*e.g.* an HGPRBMY2 with one or more functional domains or portions thereof deleted, such as Δ TM and/or Δ CD), or HGPRBMY2 fusion polypeptides
25 (*e.g.* an HGPRBMY2 or a functional domain of HGPRBMY2, such as an ECD fused to an unrelated polypeptide or peptide such as an immunoglobulin constant region, *i.e.*, IgFc), nucleic acid sequences encoding such products, and host cell expression systems that can produce such HGPRBMY2 products.

The invention also features antibodies and anti-idiotypic antibodies (including
30 Fab fragments), antagonists and agonists of the HGPRBMY2, as well as compounds or nucleic acid constructs that inhibit expression of the HGPRBMY2 gene (transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote expression of HGPRBMY2 (*e.g.*, expression
35 constructs in which HGPRBMY2 coding sequences are operatively associated with expression control elements such as promoters, promoter/enhancers, etc.). The invention also relates to host cells and animals genetically engineered to express the human

HGPRBMY2 (or mutants thereof) or to inhibit or "knock-out" expression of the animal's
5 endogenous HGPRBMY2.

The HGPRBMY2 polypeptides or peptides, HGPRBMY2 fusion polypeptides, HGPRBMY2 nucleic acid sequences, antibodies, antagonists and agonists can be useful for the detection of mutant HGPRBMY2 or inappropriately expressed HGPRBMY2 for the diagnosis of cardiovascular disorders. The HGPRBMY2 polypeptides or peptides,
10 HGPRBMY2 fusion polypeptides, HGPRBMY2 nucleic acid sequences, host cell expression systems, antibodies, antagonists, agonists and genetically engineered cells and animals can be used for screening for drugs effective in the treatment of such cardiovascular disorders. The use of engineered host cells and/or animals may offer an
15 advantage in that such systems allow not only for the identification of compounds that bind to the ECD of the HGPRBMY2, but can also identify compounds that affect the signal transduced by the activated HGPRBMY2.

Finally, the HGPRBMY2 protein products (especially soluble derivatives such as peptides corresponding to a HGPRBMY2 ECD, or truncated polypeptides lacking a
20 hydrophobic TM domain) and fusion polypeptide products (especially HGPRBMY2-Ig fusion polypeptides, *i.e.*, fusions of the HGPRBMY2 or a domain of the HGPRBMY2, e.g., ECD, Δ TM, or CD to a heterologous sequence such as IgFc), antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists or agonists (including
25 compounds that modulate signal transduction which may act on downstream targets in the HGPRBMY2 signal transduction pathway) can be used for therapy of such diseases. For example, the administration of an effective amount of a pharmaceutical composition comprising a soluble HGPRBMY2 ECD, Δ TM HGPRBMY2 or an ECD-IgFc fusion polypeptide or an anti-idiotypic antibody (or its Fab) that mimics the HGPRBMY2 ECD
30 would modulate activation of the GPCR by endogenous agonist or antagonist, and prevent or reduce binding and receptor activation, leading to heart failure.

Nucleic acid constructs encoding such HGPRBMY2 products can be used to genetically engineer host cells to express such HGPRBMY2 products *in vivo*; these
35 genetically engineered cells function in the body delivering a continuous supply of the HGPRBMY2, HGPRBMY2 peptide, soluble ECD or Δ TM or HGPRBMY2 fusion polypeptide that will modulate agonist or antagonist. Nucleic acid constructs encoding functional HGPRBMY2, mutant HGPRBMY2, as well as antisense and ribozyme

molecules can be used in "gene therapy" approaches for the modulation of HGPRBMY2
5 expression and/or activity in the treatment of cardiovascular disorders. Thus, the
invention also encompasses pharmaceutical formulations and methods for treating
cardiovascular disorders.

The invention is based, in part, on the surprising discovery of a receptor for
agonist or antagonist expressed at significant concentration in heart and thymus. Various
10 aspects of the invention are described in greater detail in the subsections below.

5.1. HGPRBMY1 Nucleic Acids

The cDNA sequence of HGPRBMY1 (SEQ ID NO:1) is 1554 base pairs long and
15 is shown in Figure 1. The first set of sequence is the 5' untranslated, the second set is the
open reading frame and the third set is the 5' untranslated. The open reading frame
extends from nucleotides 247 to 1323 of SEQ ID NO:1. The deduced amino acid
sequence encoded by the open reading frame of the cDNA of HGPRBMY1 is 359 amino
acids (SEQ ID NO:2) and is shown in Figure 2.

20 The cDNA sequence of HGPRBMY2 (SEQ ID NO:13) is 2448 base pairs long
and is shown in Figure 6. The first set of sequence is the 5' untranslated, the second set
is the open reading frame and the third set is the 5' untranslated. The open reading frame
extends from nucleotides 359 to 1651 of SEQ ID NO:13. The deduced amino acid
25 sequence encoded by the open reading frame of the cDNA of HGPRBMY2 is 431 amino
acids (SEQ ID NO:14) and is shown in Figure 7.

HGPRBMY1 nucleic acid sequences of the invention include: (a) the DNA
sequence shown in SEQ ID NO:1; (b) nucleic acid sequence that encodes the polypeptide
shown in SEQ ID NO:2; (c) any nucleic acid sequence that hybridizes to the complement
30 of the DNA sequence shown in SEQ ID NO:1 under highly stringent conditions, *e.g.*,
hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS),
1 mM EDTA at 65°C, and washing in 0.1x SSC/0.1% SDS at 68°C (Ausubel F. M. et al.,
eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates,
Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally
35 equivalent gene product; and (d) any nucleic acid sequence that hybridizes to the
complement of the DNA sequences that encode the amino acid sequence shown in SEQ
ID NO:2 contained in cDNA clone as deposited with the ATCC® under less stringent

conditions, such as moderately stringent conditions, *e.g.*, washing in 0.2x SSC/0.1% SDS at 42°C (Ausubel et al., 1989, *supra*), yet which still encodes a functionally equivalent HGPRBMY1 gene product.

HGPRBMY2 nucleic acid sequences of the invention include: (a) the DNA sequence shown in SEQ ID NO:13; (b) nucleic acid sequence that encodes the polypeptide shown in SEQ ID NO:14; (c) any nucleic acid sequence that hybridizes to the complement of the DNA sequence shown in SEQ ID NO:13 under highly stringent conditions, *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1x SSC/0.1% SDS at 68°C (Ausubel F. M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally equivalent gene product; and (d) any nucleic acid sequence that hybridizes to the complement of the DNA sequences that encode the amino acid sequence shown in SEQ ID NO:14 contained in cDNA clone as deposited with the ATCC® under less stringent conditions, such as moderately stringent conditions, *e.g.*, washing in 0.2x SSC/0.1% SDS at 42°C (Ausubel et al., 1989, *supra*), yet which still encodes a functionally equivalent HGPRBMY2 gene product.

Functional equivalents of the HGPRBMY1 include naturally occurring HGPRBMY1 present in other species, *i.e.*, orthologs, and mutant HGPRBMY1 whether naturally occurring or engineered. The invention also includes degenerate variants of sequences (a) through (d), *supra*. The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the nucleic acid sequences (a) through (d), in the preceding paragraph.

Functional equivalents of the HGPRBMY2 include naturally occurring HGPRBMY2 present in other species, *i.e.*, orthologs, and mutant HGPRBMY2 whether naturally occurring or engineered. The invention also includes degenerate variants of sequences (a) through (d), *supra*. The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the nucleic acid sequences (a) through (d), in the preceding paragraph.

Hybridization conditions may be highly stringent or less highly stringent. In instances wherein the nucleic acid molecules are deoxyoligonucleotides ("oligos"), highly stringent conditions may refer, *e.g.*, to washing in 6x SSC/0.05% sodium pyrophosphate

at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These nucleic acid molecules may encode or act as HGPRBMY1 or HGPRBMY2 antisense molecules, useful, for example, in HGPRBMY1 or HGPRBMY2 gene regulation (for and/or as antisense primers in amplification reactions of HGPRBMY1 or HGPRBMY2 gene nucleic acid sequences).

The invention features nucleic acids that are similar to the HGPRBMY1 nucleic acid sequences of the invention. A nucleic acid that has a similar sequence refers to a nucleic acid that satisfies at least one of the following: (a) a nucleic acid having a sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence of a GPCR as described herein; (b) a nucleic acid as described herein of at least 100 nucleotides, or at least 125, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1250, 1350, 1500, 1650, 1750, 1850, 2000, 2150, 2250 or 2400 contiguous nucleotides in length; and (c) a nucleic acid that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR polypeptide or peptide as described herein.

The invention features nucleic acids that are similar to the HGPRBMY2 nucleic acid sequences of the invention. A nucleic acid that has a similar sequence refers to a nucleic acid that satisfies at least one of the following: (a) a nucleic acid having a sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence of a GPCR as described herein; (b) a nucleic acid as described herein of at least 100 nucleotides, or at least 125, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1250, 1350, 1500, 1650, 1750, 1850, 2000, 2150, 2250 or 2400 contiguous nucleotides in length; and (c) a nucleic acid that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR polypeptide or peptide as described herein.

The invention also features allelic variants, *i.e.*, functional equivalents of the
5 HGPRBMY1 or HGPRBMY2 nucleic acid sequence which are naturally occurring and
appear in the same genetic locus.

Nucleic acids of HGPRBMY1 or HGPRBMY2 can also be used to identify
species orthologs of the sequence, *e.g.*, in monkeys, mice, cats, dogs, cows, fruit flies,
zebrafish or other animals. The identification of orthologs of HGPRBMY1 or
10 HGPRBMY2 in other species can be useful for developing animal model systems more
closely related to humans for purposes of drug discovery. For example, expression
libraries of cDNAs synthesized from bone marrow mRNA derived from the organism of
interest can be screened using labeled agonist derived from that species, *e.g.*, an alkaline
15 phosphatase (AP)-agonist fusion polypeptide.

Sequences of the invention may be used as part of ribozyme and/or triple helix
sequences, also useful for HGPRBMY1 gene regulation. Still further, such molecules
may be used as components of diagnostic methods whereby, for example, the presence
of a particular HGPRBMY1 allele responsible for causing an immune disorder, such as
20 immunodeficiency, may be detected.

Sequences of the invention may be used as part of ribozyme and/or triple helix
sequences, also useful for HGPRBMY2 gene regulation. Still further, such molecules
may be used as components of diagnostic methods whereby, for example, the presence
of a particular HGPRBMY2 allele responsible for causing a heart disorder, such as heart
25 failure, may be detected.

In addition to the HGPRBMY1 nucleic acid sequences described above, full
length HGPRBMY1 cDNA or gene sequences present in the same species and/or
homologues of the HGPRBMY1 gene present in other species can be identified and
30 readily isolated, without undue experimentation, by molecular biological techniques well
known in the art. The identification of homologues of HGPRBMY1 in related species can
be useful for developing animal model systems more closely related to humans for
purposes of drug discovery. For example, expression libraries of cDNAs synthesized
from spleen or bone marrow mRNA derived from the organism of interest can be
35 screened using labeled agonist derived from that species, *e.g.*, an AP-agonist fusion
polypeptide.

In addition to the HGPRBMY2 nucleic acid sequences described above, full
5 length HGPRBMY2 cDNA or gene sequences present in the same species and/or
homologues of the HGPRBMY2 gene present in other species can be identified and
readily isolated, without undue experimentation, by molecular biological techniques well
known in the art. The identification of homologues of HGPRBMY2 in related species can
10 be useful for developing animal model systems more closely related to humans for
purposes of drug discovery. For example, expression libraries of cDNAs synthesized
from heart mRNA derived from the organism of interest can be screened using labeled
agonist derived from that species, *e.g.*, an AP-agonist fusion polypeptide.

Alternatively, such cDNA libraries, or genomic DNA libraries derived from the
15 organism of interest can be screened by hybridization using the nucleic acids described
herein as hybridization or amplification probes. Furthermore, genes at other genetic loci
within the genome that encode proteins which have extensive homology to one or more
domains of the HGPRBMY1 or HGPRBMY2 gene product can also be identified via
similar techniques. In the case of cDNA libraries, such screening techniques can identify
20 clones derived from alternatively spliced transcripts in the same or different species.

Screening can be by filter hybridization, using duplicate filters. The labeled probe
can contain at least 15-30 base pairs of the HGPRBMY1 or HGPRBMY2 nucleic acid
sequence, as shown in Figure 1 or Figure 6. The hybridization washing conditions used
25 should be of a lower stringency when the cDNA library is derived from an organism
different from the type of organism from which the labeled sequence was derived. With
respect to the cloning of a human HGPRBMY1 or HGPRBMY2 homolog, using murine
HGPRBMY1 or HGPRBMY2 probes, for example, hybridization can, for example, be
performed at 65°C overnight in Church's buffer (7% SDS, 250 mM NaHPO₄, 2 mM
30 EDTA, 1% BSA). Washes can be done with 2x SSC, 0.1% SDS at 65°C and then at 0.1x
SSC, 0.1% SDS at 65°C.

Low stringency conditions are well known to those of skill in the art, and will
vary predictably depending on the specific organisms from which the library and the
35 labeled sequences are derived. For guidance regarding such conditions see, for example,
Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor
Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green
Publishing Associates and Wiley Interscience, N.Y.

Alternatively, the labeled HGPRBMY1 or HGPRBMY2 nucleic acid probe may
5 be used to screen a genomic library derived from the organism of interest, again, using
appropriately stringent conditions. The identification and characterization of human
genomic clones is helpful for designing diagnostic tests and clinical protocols for treating
cardiovascular disorders in human patients. For example, sequences derived from regions
10 adjacent to the intron/exon boundaries of the human gene can be used to design primers
for use in amplification assays to detect mutations within the exons, introns, splice sites
(*e.g.* splice acceptor and/or donor sites), etc., that can be used in diagnostics.

Further, an HGPRBMY1 or HGPRBMY2 gene homologue may be isolated from
nucleic acid of the organism of interest by performing PCR using two degenerate
15 oligonucleotide primer pools designed on the basis of amino acid sequences within the
HGPRBMY1 or HGPRBMY2 gene product disclosed herein. The template for the
reaction may be cDNA obtained by reverse transcription of mRNA prepared from, for
example, human or non-human cell lines or tissue, such as bone marrow, known or
suspected to express an HGPRBMY1 or HGPRBMY2 gene allele.

20 The PCR product may be subcloned and sequenced to ensure that the amplified
sequences represent the sequences of an HGPRBMY1 or HGPRBMY2 gene. The PCR
fragment may then be used to isolate a full length cDNA clone by a variety of methods.
For example, the amplified fragment may be labeled and used to screen a cDNA library,
25 such as a bacteriophage cDNA library. Alternatively, the labeled fragment may be used
to isolate genomic clones via the screening of a genomic library.

PCR technology may also be utilized to isolate full length cDNA sequences. For
example, RNA may be isolated, following standard procedures, from an appropriate
cellular or tissue source (*i.e.*, one known, or suspected, to express the HGPRBMY1 gene,
30 such as, for example, spleen or bone marrow). A reverse transcription reaction may be
performed on the RNA using an oligonucleotide primer specific for the most 5' end of the
amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA
hybrid may then be "tailed" with guanines using a standard terminal transferase reaction,
the hybrid may be digested with RNAase H, and second strand synthesis may then be
35 primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment
may easily be isolated. (For a review of cloning strategies which may be used, see *e.g.*,
Sambrook et al., 1989, *supra*).

PCR technology may also be utilized to isolate full length cDNA sequences. For
5 example, RNA may be isolated, following standard procedures, from an appropriate
cellular or tissue source (*i.e.*, one known, or suspected, to express the HGPRBMY2 gene,
such as, for example, heart tissues). A reverse transcription reaction may be performed
on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified
10 fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may
then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid
may be digested with RNAase H, and second strand synthesis may then be primed with
a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily
be isolated. (For a review of cloning strategies which may be used, see *e.g.*, Sambrook
15 et al., 1989, *supra*.)

The HGPRBMY1 gene sequences may additionally be used to isolate mutant
HGPRBMY1 gene alleles. Such mutant alleles may be isolated from individuals either
known or proposed to have a genotype which contributes to the symptoms of immune
disorders. Mutant alleles and mutant allele products may then be utilized in the
20 therapeutic and diagnostic systems described below. Additionally, such HGPRBMY1
gene sequences can be used to detect HGPRBMY1 gene regulatory (*e.g.*, promoter or
promotor/enhancer) defects which can affect immune function.

The HGPRBMY2 gene sequences may additionally be used to isolate mutant
25 HGPRBMY2 gene alleles. Such mutant alleles may be isolated from individuals either
known or proposed to have a genotype which contributes to the symptoms of
cardiovascular disorders. Mutant alleles and mutant allele products may then be utilized
in the therapeutic and diagnostic systems described below. Additionally, such
HGPRBMY2 gene sequences can be used to detect HGPRBMY2 gene regulatory (*e.g.*,
30 promoter or promotor/enhancer) defects which can affect cardiovascular function.

A cDNA of a mutant HGPRBMY1 or HGPRBMY2 gene may be isolated, for
example, by using PCR, a technique which is well known to those of skill in the art. In
this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT
oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an
35 individual putatively carrying the mutant HGPRBMY1 or HGPRBMY2 allele, and by
extending the new strand with reverse transcriptase. The second strand of the cDNA is
then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the

normal gene. Using these two primers, the product is then amplified via PCR, cloned into
5 a suitable vector, and subjected to DNA sequence analysis through methods well known
to those of skill in the art. By comparing the DNA sequence of the mutant HGPRBMY1
or HGPRBMY2 allele to that of the normal HGPRBMY1 or HGPRBMY2 allele, the
mutation(s) responsible for the loss or alteration of function of the mutant HGPRBMY1
or HGPRBMY2 gene product can be ascertained.

10 Alternatively, a genomic library can be constructed using DNA obtained from an
individual suspected of or known to carry the mutant HGPRBMY1 or HGPRBMY2
allele, or a cDNA library can be constructed using RNA from a tissue known, or
suspected, to express the mutant HGPRBMY1 or HGPRBMY2 allele. The normal
15 HGPRBMY1 or HGPRBMY2 gene or any suitable fragment thereof may then be labeled
and used as a probe to identify the corresponding mutant HGPRBMY1 or HGPRBMY2
allele in such libraries. Clones containing the mutant HGPRBMY1 or HGPRBMY2 gene
sequences may then be purified and subjected to sequence analysis according to methods
well known to those of skill in the art.

20 Additionally, an expression library can be constructed utilizing cDNA
synthesized from, for example, RNA isolated from a tissue known, or suspected, to
express a mutant HGPRBMY1 or HGPRBMY2 allele in an individual suspected of or
known to carry such a mutant allele. In this manner, gene products made by the putatively
25 mutant tissue may be expressed and screened using standard antibody screening
techniques in conjunction with antibodies raised against the normal HGPRBMY1 or
HGPRBMY2 gene product, as described, below, in Section 5.3. (For screening
techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory
Manual", Cold Spring Harbor Press, Cold Spring Harbor.) Additionally, screening can
30 be accomplished by screening with labeled agonist or antagonist fusion polypeptides,
such as, for example, AP-GPCR or GPCR-AP fusion polypeptides. In cases where an
HGPRBMY1 or HGPRBMY2 mutation results in an expressed gene product with altered
function (*e.g.*, as a result of a missense or a frameshift mutation), a polyclonal set of
antibodies to HGPRBMY1 or HGPRBMY2 are likely to cross-react with the mutant
35 HGPRBMY1 or HGPRBMY2 gene product. Library clones detected via their reaction
with such labeled antibodies can be purified and subjected to sequence analysis according
to methods well known to those of skill in the art.

HGPRBMY1 or HGPRBMY2 nucleic acids can also be utilized for chromosomal
5 mapping, or as chromosomal markers, *e.g.*, in radiation hybrid mapping.

The invention also features identifying detecting or diagnosing cells or tissues
which express a mRNA or HGPRBMY1 or HGPRBMY2. The invention also features
nucleic acid sequences that encode mutant HGPRBMY1 or HGPRBMY2 polypeptides,
10 peptides of the HGPRBMY1 or HGPRBMY2, truncated HGPRBMY1 or HGPRBMY2,
and HGPRBMY1 or HGPRBMY2 fusion polypeptides. These include, but are not limited
to nucleic acid sequences encoding mutant HGPRBMY1 or HGPRBMY2 described in
section 5.2 *infra*; polypeptides or peptides corresponding to the ECD, TM and/or CD
domains of the HGPRBMY1 or HGPRBMY2 or portions of these domains; truncated
15 HGPRBMY1 or HGPRBMY2 in which one or two of the domains are deleted, *e.g.*, a
soluble HGPRBMY1 or HGPRBMY2 lacking the TM or both the TM and CD regions,
or a truncated, nonfunctional HGPRBMY1 or HGPRBMY2 lacking all or a portion of
the CD region. Nucleotides encoding fusion polypeptides may include by are not limited
to full length HGPRBMY1 or HGPRBMY2, HGPRBMY1 or HGPRBMY2 peptides, or
20 HGPRBMY1 or HGPRBMY2 polypeptides or peptides fused to an unrelated polypeptide
or peptide, such as for example, a transmembrane sequence, which anchors the
HGPRBMY1 or HGPRBMY2 ECD to the cell membrane; an Ig-Fc domain which
increases the stability and half life of the resulting fusion polypeptide (*e.g.*, HGPRBMY1
or HGPRBMY2-Ig) in the bloodstream; or an enzyme, fluorescent polypeptide,
25 luminescent polypeptide which can be used as a marker.

The invention also encompasses (a) DNA vectors that contain any of the
foregoing HGPRBMY1 or HGPRBMY2 coding sequences and/or their complements
(*i.e.*, antisense); (b) DNA expression vectors that contain any of the foregoing
30 HGPRBMY1 or HGPRBMY2 coding sequences operatively associated with a regulatory
element that directs the expression of the coding sequences; and (c) genetically
engineered host cells that contain any of the foregoing HGPRBMY1 or HGPRBMY2
coding sequences operatively associated with a regulatory element that directs the
expression of the coding sequences in the host cell.

35 As used herein, regulatory elements include but are not limited to inducible and
non-inducible promoters, enhancers, operators and other elements known to those skilled
in the art that drive and regulate expression. Such regulatory elements include but are not

limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters
5 of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the
major operator and promoter regions of phage A, the control regions of fd coat
polypeptide, the promoter for 3-phosphoglycerate kinase, the promoters of acid
phosphatase, and the promoters of the yeast \square -mating factors.

These expression and cloning methods include, for example, *in vitro* recombinant
10 DNA techniques, synthetic techniques, and *in vivo* genetic recombination. (For example,
Sambrook et al., 1989, supra, and Ausubel et al., 1989, supra). Alternatively, RNA
capable of encoding HGPRBMY1 nucleic acid sequences may be chemically synthesized
using, for example, synthesizers. See, for example, the techniques described in
15 "Oligonucleotide Synthesis", 1984, Gait, M. J. ed., IRL Press, Oxford, which is
incorporated by reference herein in its entirety.

As referenced elsewhere herein, characterization of the HGPRBMY1 polypeptide
of the present invention led to the determination that it is involved in the modulation of
the cyclin p27 protein, in addition to, the apoptosis regulatory protein I κ B, either directly
20 or indirectly.

In preferred embodiments, HGPRBMY1 polynucleotides and polypeptides,
including fragments thereof, are useful for treating, diagnosing, and/or ameliorating cell
cycle defects, disorders related to aberrant phosphorylation, disorders related to aberrant
25 signal transduction, proliferating disorders, and/or cancers.

In preferred embodiments, antagonists directed to HGPRBMY1 are useful for
decreasing cellular proliferation, decreasing cellular proliferation in rapidly proliferating
cells, increasing the number of cells in the G1 phase of the cell cycle, and decreasing the
number of cells that progress to the S phase of the cell cycle.

Moreover, agonists directed against HGPRBMY1 are useful for increasing
cellular proliferation, increasing cellular proliferation in rapidly proliferating cells,
decreasing the number of cells in the G1 phase of the cell cycle, and increasing the
number of cells that progress to the S phase of the cell cycle. Such agonists would be
particularly useful for transforming normal cells into immortalized cell lines, stimulating
35 hematopoietic cells to grow and divide, increasing recovery rates of cancer patients that
have undergone chemotherapy or other therapeutic regimen, by boosting their immune
responses, etc.

In preferred embodiments, HGPRBMY1 polynucleotides and polypeptides, including fragments thereof, are useful for treating, diagnosing, and/or ameliorating proliferative disorders, cancers, ischemia-reperfusion injury, heart failure, immuno compromised conditions, HIV infection, and renal diseases.

Moreover, HGPRBMY1 polynucleotides and polypeptides, including fragments thereof, are useful for increasing NF-kB activity, decreasing apoptotic events, and/or decreasing I κ B expression or activity levels.

In preferred embodiments, antagonists directed against HGPRBMY1 are useful for treating, diagnosing, and/or ameliorating autoimmune disorders, disorders related to hyper immune activity, inflammatory conditions, disorders related to aberrant acute phase responses, hypercongenital conditions, birth defects, necrotic lesions, wounds, organ transplant rejection, conditions related to organ transplant rejection, disorders related to aberrant signal transduction, proliferating disorders, cancers, HIV, and HIV propagation in cells infected with other viruses.

Moreover, antagonists directed against HGPRBMY1 are useful for decreasing NF-kB activity, increasing apoptotic events, and/or increasing I κ B expression or activity levels.

In preferred embodiments, agonists directed against HGPRBMY1 are useful for treating, diagnosing, and/or ameliorating autoimmune disorders, disorders related to hyper immune activity, hypercongenital conditions, birth defects, necrotic lesions, wounds, disorders related to aberrant signal transduction, immuno compromised conditions, HIV infection, proliferating disorders, Alzheimer's, and/or cancers.

Moreover, agonists directed against HGPRBMY1 are useful for increasing NF-kB activity, decreasing apoptotic events, and/or decreasing I κ B expression or activity levels.

5.2. HGPRBMY1 and HGPRBMY2 Polypeptides

The term "peptides" as used herein is meant to comprise a small number of amino acids connected by peptide bonds. The term "polypeptide" generally refers to longer chains of amino acids but does not refer to a specific length, thus as used herein, polypeptides include proteins (a term usually reserved for a functional unit which may consist of either a single polypeptide or several polypeptides).

The invention features polypeptides and/or peptides that are similar to the
5 sequence of HGPRBMY1. A polypeptide or peptide that has a similar amino acid
sequence refers to a polypeptide or peptide sequence that satisfies at least one of the
following: (a) a polypeptide having an amino acid sequence that is at least 40%, at least
45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%,
10 at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the
amino acid sequence of a GPCR polypeptide or peptide as described herein; (b) a
polypeptide or peptide encoded by a nucleic acid sequence that hybridizes under stringent
conditions to a nucleic acid sequence encoding a GPCR as described herein of at least 20
amino acid residues, at least 25, at least 40, at least 50, at least 60, at least 70, at least 80,
15 at least 90, at least 100, at least 125, at least 150, at least 200, at least 225, at least 250,
at least 275, at least 300 or at least 350 amino acids; and (c) a polypeptide encoded by a
nucleic acid sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least
60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%,
at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR
20 polypeptide or peptide as described herein.

The invention features polypeptides and/or peptides that are similar to the
sequence of HGPRBMY2. A polypeptide or peptide that has a similar amino acid
sequence refers to a polypeptide or peptide sequence that satisfies at least one of the
25 following: (a) a polypeptide having an amino acid sequence that is at least 40%, at least
45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%,
at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the
amino acid sequence of a GPCR polypeptide or peptide as described herein; (b) a
polypeptide or peptide encoded by a nucleic acid sequence that hybridizes under stringent
30 conditions to a nucleic acid sequence encoding a GPCR as described herein of at least 20
amino acid residues, at least 25, at least 40, at least 50, at least 60, at least 70, at least 80,
at least 90, at least 100, at least 125, at least 150, at least 200, at least 225, at least 250,
at least 275, at least 300 or at least 350 amino acids; and (c) a polypeptide encoded by a
nucleic acid sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least
35 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%,
at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR
polypeptide or peptide as described herein.

A polypeptide with similar structure and/or function to a GPCR polypeptide or
5 as described herein refers to a polypeptide that has a similar secondary, tertiary or
quaternary structure of a GPCR polypeptide, *e.g.*, a protein or a fusion protein, as
described herein. The structure of a polypeptide can be determined by methods known to
those skilled in the art, including but not limited to, X-ray crystallography, nuclear
magnetic resonance, and crystallographic electron microscopy.

10 HGPRBMY1 polypeptides and peptides, mutations, truncations and/or
HGPRBMY1 fusion polypeptides of any of the foregoing can be used for, but not limited
to, the generation of antibodies, as reagents in diagnostic assays, the identification of
other cellular gene products involved in the regulation of immune function, as reagents
15 in assays for screening for compounds that can be used in the treatment of immune
disorders, and as pharmaceutical reagents useful in the treatment of immune disorders
related to the HGPRBMY1.

In preferred embodiments, the following N-terminal HGPRBMY1 deletion
polypeptides are encompassed by the present invention: M1-F359, Q2-F359, V3-F359,
20 P4-F359, N5-F359, S6-F359, T7-F359, G8-F359, P9-F359, D10-F359, N11-F359, A12-
F359, T13-F359, L14-F359, Q15-F359, M16-F359, L17-F359, R18-F359, N19-F359,
P20-F359, A21-F359, I22-F359, A23-F359, V24-F359, A25-F359, L26-F359, P27-F359,
V28-F359, V29-F359, Y30-F359, S31-F359, L32-F359, V33-F359, A34-F359, A35-
25 F359, V36-F359, S37-F359, I38-F359, P39-F359, G40-F359, N41-F359, L42-F359, F43-
F359, S44-F359, L45-F359, W46-F359, V47-F359, L48-F359, C49-F359, R50-F359,
R51-F359, M52-F359, G53-F359, P54-F359, R55-F359, S56-F359, P57-F359, S58-
F359, V59-F359, I60-F359, F61-F359, M62-F359, I63-F359, N64-F359, L65-F359, S66-
F359, V67-F359, T68-F359, D69-F359, L70-F359, M71-F359, L72-F359, A73-F359,
30 S74-F359, V75-F359, L76-F359, P77-F359, F78-F359, Q79-F359, I80-F359, Y81-F359,
Y82-F359, H83-F359, C84-F359, N85-F359, R86-F359, H87-F359, H88-F359, W89-
F359, V90-F359, F91-F359, G92-F359, V93-F359, L94-F359, L95-F359, C96-F359,
N97-F359, V98-F359, V99-F359, T100-F359, V101-F359, A102-F359, F103-F359,
Y104-F359, A105-F359, N106-F359, M107-F359, Y108-F359, S109-F359, S110-F359,
35 I111-F359, L112-F359, T113-F359, M114-F359, T115-F359, C116-F359, I117-F359,
S118-F359, V119-F359, E120-F359, R121-F359, F122-F359, L123-F359, G124-F359,
V125-F359, L126-F359, Y127-F359, P128-F359, L129-F359, S130-F359, S131-F359,

K132-F359, R133-F359, W134-F359, R135-F359, R136-F359, R137-F359, R138-F359,
5 Y139-F359, A140-F359, V141-F359, A142-F359, A143-F359, C144-F359, A145-F359,
G146-F359, T147-F359, W148-F359, L149-F359, L150-F359, L151-F359, L152-F359,
T153-F359, A154-F359, L155-F359, S156-F359, P157-F359, L158-F359, A159-F359,
R160-F359, T161-F359, D162-F359, L163-F359, T164-F359, Y165-F359, P166-F359,
10 V167-F359, H168-F359, A169-F359, L170-F359, G171-F359, I172-F359, I173-F359,
T174-F359, C175-F359, F176-F359, D177-F359, V178-F359, L179-F359, K180-F359,
W181-F359, T182-F359, M183-F359, L184-F359, P185-F359, S186-F359, V187-F359,
A188-F359, M189-F359, W190-F359, A191-F359, V192-F359, F193-F359, L194-F359,
F195-F359, T196-F359, I197-F359, F198-F359, I199-F359, L200-F359, L201-F359,
15 F202-F359, L203-F359, I204-F359, P205-F359, F206-F359, V207-F359, I208-F359,
T209-F359, V210-F359, A211-F359, C212-F359, Y213-F359, T214-F359, A215-F359,
T216-F359, I217-F359, L218-F359, K219-F359, L220-F359, L221-F359, R222-F359,
T223-F359, E224-F359, E225-F359, A226-F359, H227-F359, G228-F359, R229-F359,
E230-F359, Q231-F359, R232-F359, R233-F359, R234-F359, A235-F359, V236-F359,
20 G237-F359, L238-F359, A239-F359, A240-F359, V241-F359, V242-F359, L243-F359,
L244-F359, A245-F359, F246-F359, V247-F359, T248-F359, C249-F359, F250-F359,
A251-F359, P252-F359, N253-F359, N254-F359, F255-F359, V256-F359, L257-F359,
L258-F359, A259-F359, H260-F359, I261-F359, V262-F359, S263-F359, R264-F359,
25 L265-F359, F266-F359, Y267-F359, G268-F359, K269-F359, S270-F359, Y271-F359,
Y272-F359, H273-F359, V274-F359, Y275-F359, K276-F359, L277-F359, T278-F359,
L279-F359, C280-F359, L281-F359, S282-F359, C283-F359, L284-F359, N285-F359,
N286-F359, C287-F359, L288-F359, D289-F359, P290-F359, F291-F359, V292-F359,
Y293-F359, Y294-F359, F295-F359, A296-F359, S297-F359, R298-F359, E299-F359,
30 F300-F359, Q301-F359, L302-F359, R303-F359, L304-F359, R305-F359, E306-F359,
Y307-F359, L308-F359, G309-F359, C310-F359, R311-F359, R312-F359, V313-F359,
P314-F359, R315-F359, D316-F359, T317-F359, L318-F359, D319-F359, T320-F359,
R321-F359, R322-F359, E323-F359, S324-F359, L325-F359, F326-F359, S327-F359,
A328-F359, R329-F359, T330-F359, T331-F359, S332-F359, V333-F359, R334-F359,
35 S335-F359, E336-F359, A337-F359, G338-F359, A339-F359, H340-F359, P341-F359,
E342-F359, G343-F359, M344-F359, E345-F359, G346-F359, A347-F359, T348-F359,
R349-F359, P350-F359, G351-F359, L352-F359, and/or Q353-F359 of SEQ ID NO:2.

Polynucleotide sequences encoding these polypeptides are also provided. The present
5 invention also encompasses the use of these N-terminal HGPRBMY1 deletion
polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal HGPRBMY1 deletion
polypeptides are encompassed by the present invention: M1-F359, M1-V358, M1-S357,
M1-E356, M1-Q355, M1-R354, M1-Q353, M1-L352, M1-G351, M1-P350, M1-R349,
10 M1-T348, M1-A347, M1-G346, M1-E345, M1-M344, M1-G343, M1-E342, M1-P341,
M1-H340, M1-A339, M1-G338, M1-A337, M1-E336, M1-S335, M1-R334, M1-V333,
M1-S332, M1-T331, M1-T330, M1-R329, M1-A328, M1-S327, M1-F326, M1-L325,
M1-S324, M1-E323, M1-R322, M1-R321, M1-T320, M1-D319, M1-L318, M1-T317,
15 M1-D316, M1-R315, M1-P314, M1-V313, M1-R312, M1-R311, M1-C310, M1-G309,
M1-L308, M1-Y307, M1-E306, M1-R305, M1-L304, M1-R303, M1-L302, M1-Q301,
M1-F300, M1-E299, M1-R298, M1-S297, M1-A296, M1-F295, M1-Y294, M1-Y293,
M1-V292, M1-F291, M1-P290, M1-D289, M1-L288, M1-C287, M1-N286, M1-N285,
M1-L284, M1-C283, M1-S282, M1-L281, M1-C280, M1-L279, M1-T278, M1-L277,
20 M1-K276, M1-Y275, M1-V274, M1-H273, M1-Y272, M1-Y271, M1-S270, M1-K269,
M1-G268, M1-Y267, M1-F266, M1-L265, M1-R264, M1-S263, M1-V262, M1-I261,
M1-H260, M1-A259, M1-L258, M1-L257, M1-V256, M1-F255, M1-N254, M1-N253,
M1-P252, M1-A251, M1-F250, M1-C249, M1-T248, M1-V247, M1-F246, M1-A245,
25 M1-L244, M1-L243, M1-V242, M1-V241, M1-A240, M1-A239, M1-L238, M1-G237,
M1-V236, M1-A235, M1-R234, M1-R233, M1-R232, M1-Q231, M1-E230, M1-R229,
M1-G228, M1-H227, M1-A226, M1-E225, M1-E224, M1-T223, M1-R222, M1-L221,
M1-L220, M1-K219, M1-L218, M1-I217, M1-T216, M1-A215, M1-T214, M1-Y213,
M1-C212, M1-A211, M1-V210, M1-T209, M1-I208, M1-V207, M1-F206, M1-P205,
30 M1-I204, M1-L203, M1-F202, M1-L201, M1-L200, M1-I199, M1-F198, M1-I197, M1-
T196, M1-F195, M1-L194, M1-F193, M1-V192, M1-A191, M1-W190, M1-M189, M1-
A188, M1-V187, M1-S186, M1-P185, M1-L184, M1-M183, M1-T182, M1-W181, M1-
K180, M1-L179, M1-V178, M1-D177, M1-F176, M1-C175, M1-T174, M1-I173, M1-
I172, M1-G171, M1-L170, M1-A169, M1-H168, M1-V167, M1-P166, M1-Y165, M1-
35 T164, M1-L163, M1-D162, M1-T161, M1-R160, M1-A159, M1-L158, M1-P157, M1-
S156, M1-L155, M1-A154, M1-T153, M1-L152, M1-L151, M1-L150, M1-L149, M1-
W148, M1-T147, M1-G146, M1-A145, M1-C144, M1-A143, M1-A142, M1-V141, M1-

A140, M1-Y139, M1-R138, M1-R137, M1-R136, M1-R135, M1-W134, M1-R133, M1-
 5 K132, M1-S131, M1-S130, M1-L129, M1-P128, M1-Y127, M1-L126, M1-V125, M1-
 G124, M1-L123, M1-F122, M1-R121, M1-E120, M1-V119, M1-S118, M1-I117, M1-
 C116, M1-T115, M1-M114, M1-T113, M1-L112, M1-I111, M1-S110, M1-S109, M1-
 Y108, M1-M107, M1-N106, M1-A105, M1-Y104, M1-F103, M1-A102, M1-V101, M1-
 10 T100, M1-V99, M1-V98, M1-N97, M1-C96, M1-L95, M1-L94, M1-V93, M1-G92, M1-
 F91, M1-V90, M1-W89, M1-H88, M1-H87, M1-R86, M1-N85, M1-C84, M1-H83, M1-
 Y82, M1-Y81, M1-I80, M1-Q79, M1-F78, M1-P77, M1-L76, M1-V75, M1-S74, M1-
 A73, M1-L72, M1-M71, M1-L70, M1-D69, M1-T68, M1-V67, M1-S66, M1-L65, M1-
 N64, M1-I63, M1-M62, M1-F61, M1-I60, M1-V59, M1-S58, M1-P57, M1-S56, M1-
 15 R55, M1-P54, M1-G53, M1-M52, M1-R51, M1-R50, M1-C49, M1-L48, M1-V47, M1-
 W46, M1-L45, M1-S44, M1-F43, M1-L42, M1-N41, M1-G40, M1-P39, M1-I38, M1-
 S37, M1-V36, M1-A35, M1-A34, M1-V33, M1-L32, M1-S31, M1-Y30, M1-V29, M1-
 V28, M1-P27, M1-L26, M1-A25, M1-V24, M1-A23, M1-I22, M1-A21, M1-P20, M1-
 N19, M1-R18, M1-L17, M1-M16, M1-Q15, M1-L14, M1-T13, M1-A12, M1-N11, M1-
 20 D10, M1-P9, M1-G8, and/or M1-T7 of SEQ ID NO:2. Polynucleotide sequences
 encoding these polypeptides are also provided. The present invention also encompasses
 the use of these C-terminal HGPRBMY1 deletion polypeptides as immunogenic and/or
 antigenic epitopes as described elsewhere herein.

25 HGPRBMY2 polypeptides and peptides, mutated, truncated or deleted forms of
 the HGPRBMY2 and/or HGPRBMY2 fusion polypeptides can be prepared for a variety
 of uses, including but not limited to the generation of antibodies, as reagents in diagnostic
 assays, the identification of other cellular gene products involved in the regulation of
 cardiovascular, as reagents in assays for screening for compounds that can be used in the
 30 treatment of cardiovascular disorders, and as pharmaceutical reagents useful in the
 treatment of cardiovascular disorders related to the HGPRBMY2.

The deduced amino acid sequence encoded by the open reading frame of
 HGPRBMY2 is 431 amino acids (SEQ ID NO:14) and is shown in Figure 7. The
 extracellular domains ("ECD") of HGPRBMY2 extend from about amino acid residues
 1 to about 45, about 105 to about 119, about 182 to about 212, and about 293 to about
 35 311 of SEQ ID NO:14; the transmembrane domains of HGPRBMY2 extend from about
 amino acid residues 46 to about 69, about 82 to about 104, about 119 to about 141, about

162 to about 181, about 213 to about 233, about 272 to about 292, and about 312 to about
5 335 of SEQ ID NO:14; and the cytoplasmic domains of HGPRBMY2 extend from about
amino acid residue 69 to about 81, about 142 to about 161, about 234 to about 271, and
about 336 to about 431 of SEQ ID NO:14.

In preferred embodiments, the following N-terminal HGPRBMY2 deletion
polypeptides are encompassed by the present invention: M1-H431, Q2-H431, A3-H431,
10 L4-H431, N5-H431, I6-H431, T7-H431, P8-H431, E9-H431, Q10-H431, F11-H431,
S12-H431, R13-H431, L14-H431, L15-H431, R16-H431, D17-H431, H18-H431, N19-
H431, L20-H431, T21-H431, R22-H431, E23-H431, Q24-H431, F25-H431, I26-H431,
A27-H431, L28-H431, Y29-H431, R30-H431, L31-H431, R32-H431, P33-H431, L34-
15 H431, V35-H431, Y36-H431, T37-H431, P38-H431, E39-H431, L40-H431, P41-H431,
G42-H431, R43-H431, A44-H431, K45-H431, L46-H431, A47-H431, L48-H431, V49-
H431, L50-H431, T51-H431, G52-H431, V53-H431, L54-H431, I55-H431, F56-H431,
A57-H431, L58-H431, A59-H431, L60-H431, F61-H431, G62-H431, N63-H431, A64-
H431, L65-H431, V66-H431, F67-H431, Y68-H431, V69-H431, V70-H431, T71-H431,
20 R72-H431, S73-H431, K74-H431, A75-H431, M76-H431, R77-H431, T78-H431, V79-
H431, T80-H431, N81-H431, I82-H431, F83-H431, I84-H431, C85-H431, S86-H431,
L87-H431, A88-H431, L89-H431, S90-H431, D91-H431, L92-H431, L93-H431, I94-
H431, T95-H431, F96-H431, F97-H431, C98-H431, I99-H431, P100-H431, V101-H431,
25 T102-H431, M103-H431, L104-H431, Q105-H431, N106-H431, I107-H431, S108-
H431, D109-H431, N110-H431, W111-H431, L112-H431, G113-H431, G114-H431,
A115-H431, F116-H431, I117-H431, C118-H431, K119-H431, M120-H431, V121-
H431, P122-H431, F123-H431, V124-H431, Q125-H431, S126-H431, T127-H431,
A128-H431, V129-H431, V130-H431, T131-H431, E132-H431, I133-H431, L134-H431,
30 T135-H431, M136-H431, T137-H431, C138-H431, I139-H431, A140-H431, V141-
H431, E142-H431, R143-H431, H144-H431, Q145-H431, G146-H431, L147-H431,
V148-H431, H149-H431, P150-H431, F151-H431, K152-H431, M153-H431, K154-
H431, W155-H431, Q156-H431, Y157-H431, T158-H431, N159-H431, R160-H431,
R161-H431, A162-H431, F163-H431, T164-H431, M165-H431, L166-H431, G167-
35 H431, V168-H431, V169-H431, W170-H431, L171-H431, V172-H431, A173-H431,
V174-H431, I175-H431, V176-H431, G177-H431, S178-H431, P179-H431, M180-
H431, W181-H431, H182-H431, V183-H431, Q184-H431, Q185-H431, L186-H431,

E187-H431, I188-H431, K189-H431, Y190-H431, D191-H431, F192-H431, L193-H431,
5 Y194-H431, E195-H431, K196-H431, E197-H431, H198-H431, I199-H431, C200-
H431, C201-H431, L202-H431, E203-H431, E204-H431, W205-H431, T206-H431,
S207-H431, P208-H431, V209-H431, H210-H431, Q211-H431, K212-H431, I213-H431,
Y214-H431, T215-H431, T216-H431, F217-H431, I218-H431, L219-H431, V220-H431,
10 I221-H431, L222-H431, F223-H431, L224-H431, L225-H431, P226-H431, L227-H431,
M228-H431, V229-H431, M230-H431, L231-H431, I232-H431, L233-H431, Y234-
H431, S235-H431, K236-H431, I237-H431, G238-H431, Y239-H431, E240-H431,
L241-H431, W242-H431, I243-H431, K244-H431, K245-H431, R246-H431, V247-
H431, G248-H431, D249-H431, G250-H431, S251-H431, V252-H431, L253-H431,
15 R254-H431, T255-H431, I256-H431, H257-H431, G258-H431, K259-H431, E260-
H431, M261-H431, S262-H431, K263-H431, I264-H431, A265-H431, R266-H431,
K267-H431, K268-H431, K269-H431, R270-H431, A271-H431, V272-H431, I273-
H431, M274-H431, M275-H431, V276-H431, T277-H431, V278-H431, V279-H431,
A280-H431, L281-H431, F282-H431, A283-H431, V284-H431, C285-H431, W286-
20 H431, A287-H431, P288-H431, F289-H431, H290-H431, V291-H431, V292-H431,
H293-H431, M294-H431, M295-H431, I296-H431, E297-H431, Y298-H431, S299-
H431, N300-H431, F301-H431, E302-H431, K303-H431, E304-H431, Y305-H431,
D306-H431, D307-H431, V308-H431, T309-H431, I310-H431, K311-H431, M312-
25 H431, I313-H431, F314-H431, A315-H431, I316-H431, V317-H431, Q318-H431, I319-
H431, I320-H431, G321-H431, F322-H431, S323-H431, N324-H431, S325-H431, I326-
H431, C327-H431, N328-H431, P329-H431, I330-H431, V331-H431, Y332-H431,
A333-H431, F334-H431, M335-H431, N336-H431, E337-H431, N338-H431, F339-
H431, K340-H431, K341-H431, N342-H431, V343-H431, L344-H431, S345-H431,
30 A346-H431, V347-H431, C348-H431, Y349-H431, C350-H431, I351-H431, V352-
H431, N353-H431, K354-H431, T355-H431, F356-H431, S357-H431, P358-H431,
A359-H431, Q360-H431, R361-H431, H362-H431, G363-H431, N364-H431, S365-
H431, G366-H431, I367-H431, T368-H431, M369-H431, M370-H431, R371-H431,
K372-H431, K373-H431, A374-H431, K375-H431, F376-H431, S377-H431, L378-
35 H431, R379-H431, E380-H431, N381-H431, P382-H431, V383-H431, E384-H431,
E385-H431, T386-H431, K387-H431, G388-H431, E389-H431, A390-H431, F391-
H431, S392-H431, D393-H431, G394-H431, N395-H431, I396-H431, E397-H431,

V398-H431, K399-H431, L400-H431, C401-H431, E402-H431, Q403-H431, T404-
5 H431, E405-H431, E406-H431, K407-H431, K408-H431, K409-H431, L410-H431,
K411-H431, R412-H431, H413-H431, L414-H431, A415-H431, L416-H431, F417-
H431, R418-H431, S419-H431, E420-H431, L421-H431, A422-H431, E423-H431,
N424-H431, and/or S425-H431 of SEQ ID NO:14. Polynucleotide sequences encoding
10 these polypeptides are also provided. The present invention also encompasses the use of
these N-terminal HGPRBMY2 deletion polypeptides as immunogenic and/or antigenic
epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal HGPRBMY2 deletion
polypeptides are encompassed by the present invention: M1-H431, M1-G430, M1-S429,
15 M1-D428, M1-L427, M1-P426, M1-S425, M1-N424, M1-E423, M1-A422, M1-L421,
M1-E420, M1-S419, M1-R418, M1-F417, M1-L416, M1-A415, M1-L414, M1-H413,
M1-R412, M1-K411, M1-L410, M1-K409, M1-K408, M1-K407, M1-E406, M1-E405,
M1-T404, M1-Q403, M1-E402, M1-C401, M1-L400, M1-K399, M1-V398, M1-E397,
M1-I396, M1-N395, M1-G394, M1-D393, M1-S392, M1-F391, M1-A390, M1-E389,
20 M1-G388, M1-K387, M1-T386, M1-E385, M1-E384, M1-V383, M1-P382, M1-N381,
M1-E380, M1-R379, M1-L378, M1-S377, M1-F376, M1-K375, M1-A374, M1-K373,
M1-K372, M1-R371, M1-M370, M1-M369, M1-T368, M1-I367, M1-G366, M1-S365,
M1-N364, M1-G363, M1-H362, M1-R361, M1-Q360, M1-A359, M1-P358, M1-S357,
25 M1-F356, M1-T355, M1-K354, M1-N353, M1-V352, M1-I351, M1-C350, M1-Y349,
M1-C348, M1-V347, M1-A346, M1-S345, M1-L344, M1-V343, M1-N342, M1-K341,
M1-K340, M1-F339, M1-N338, M1-E337, M1-N336, M1-M335, M1-F334, M1-A333,
M1-Y332, M1-V331, M1-I330, M1-P329, M1-N328, M1-C327, M1-I326, M1-S325,
M1-N324, M1-S323, M1-F322, M1-G321, M1-I320, M1-I319, M1-Q318, M1-V317,
30 M1-I316, M1-A315, M1-F314, M1-I313, M1-M312, M1-K311, M1-I310, M1-T309, M1-
V308, M1-D307, M1-D306, M1-Y305, M1-E304, M1-K303, M1-E302, M1-F301, M1-
N300, M1-S299, M1-Y298, M1-E297, M1-I296, M1-M295, M1-M294, M1-H293, M1-
V292, M1-V291, M1-H290, M1-F289, M1-P288, M1-A287, M1-W286, M1-C285, M1-
V284, M1-A283, M1-F282, M1-L281, M1-A280, M1-V279, M1-V278, M1-T277, M1-
35 V276, M1-M275, M1-M274, M1-I273, M1-V272, M1-A271, M1-R270, M1-K269, M1-
K268, M1-K267, M1-R266, M1-A265, M1-I264, M1-K263, M1-S262, M1-M261, M1-
E260, M1-K259, M1-G258, M1-H257, M1-I256, M1-T255, M1-R254, M1-L253, M1-

V252, M1-S251, M1-G250, M1-D249, M1-G248, M1-V247, M1-R246, M1-K245, M1-
5 K244, M1-I243, M1-W242, M1-L241, M1-E240, M1-Y239, M1-G238, M1-I237, M1-
K236, M1-S235, M1-Y234, M1-L233, M1-I232, M1-L231, M1-M230, M1-V229, M1-
M228, M1-L227, M1-P226, M1-L225, M1-L224, M1-F223, M1-L222, M1-I221, M1-
V220, M1-L219, M1-I218, M1-F217, M1-T216, M1-T215, M1-Y214, M1-I213, M1-
10 K212, M1-Q211, M1-H210, M1-V209, M1-P208, M1-S207, M1-T206, M1-W205, M1-
E204, M1-E203, M1-L202, M1-C201, M1-C200, M1-I199, M1-H198, M1-E197, M1-
K196, M1-E195, M1-Y194, M1-L193, M1-F192, M1-D191, M1-Y190, M1-K189, M1-
I188, M1-E187, M1-L186, M1-Q185, M1-Q184, M1-V183, M1-H182, M1-W181, M1-
M180, M1-P179, M1-S178, M1-G177, M1-V176, M1-I175, M1-V174, M1-A173, M1-
15 V172, M1-L171, M1-W170, M1-V169, M1-V168, M1-G167, M1-L166, M1-M165, M1-
T164, M1-F163, M1-A162, M1-R161, M1-R160, M1-N159, M1-T158, M1-Y157, M1-
Q156, M1-W155, M1-K154, M1-M153, M1-K152, M1-F151, M1-P150, M1-H149, M1-
V148, M1-L147, M1-G146, M1-Q145, M1-H144, M1-R143, M1-E142, M1-V141, M1-
A140, M1-I139, M1-C138, M1-T137, M1-M136, M1-T135, M1-L134, M1-I133, M1-
20 E132, M1-T131, M1-V130, M1-V129, M1-A128, M1-T127, M1-S126, M1-Q125, M1-
V124, M1-F123, M1-P122, M1-V121, M1-M120, M1-K119, M1-C118, M1-I117, M1-
F116, M1-A115, M1-G114, M1-G113, M1-L112, M1-W111, M1-N110, M1-D109, M1-
S108, M1-I107, M1-N106, M1-Q105, M1-L104, M1-M103, M1-T102, M1-V101, M1-
25 P100, M1-I99, M1-C98, M1-F97, M1-F96, M1-T95, M1-I94, M1-L93, M1-L92, M1-
D91, M1-S90, M1-L89, M1-A88, M1-L87, M1-S86, M1-C85, M1-I84, M1-F83, M1-I82,
M1-N81, M1-T80, M1-V79, M1-T78, M1-R77, M1-M76, M1-A75, M1-K74, M1-S73,
M1-R72, M1-T71, M1-V70, M1-V69, M1-Y68, M1-F67, M1-V66, M1-L65, M1-A64,
M1-N63, M1-G62, M1-F61, M1-L60, M1-A59, M1-L58, M1-A57, M1-F56, M1-I55,
30 M1-L54, M1-V53, M1-G52, M1-T51, M1-L50, M1-V49, M1-L48, M1-A47, M1-L46,
M1-K45, M1-A44, M1-R43, M1-G42, M1-P41, M1-L40, M1-E39, M1-P38, M1-T37,
M1-Y36, M1-V35, M1-L34, M1-P33, M1-R32, M1-L31, M1-R30, M1-Y29, M1-L28,
M1-A27, M1-I26, M1-F25, M1-Q24, M1-E23, M1-R22, M1-T21, M1-L20, M1-N19,
M1-H18, M1-D17, M1-R16, M1-L15, M1-L14, M1-R13, M1-S12, M1-F11, M1-Q10,
35 M1-E9, M1-P8, and/or M1-T7 of SEQ ID NO: 14. Polynucleotide sequences encoding
these polypeptides are also provided. The present invention also encompasses the use of

these C-terminal HGPRBMY2 deletion polypeptides as immunogenic and/or antigenic
 5 epitopes as described elsewhere herein.

Figure 8 depicts the putative transmembrane regions of the HGPRBMY2
 polypeptide as shaded areas of the sequence, and also presents a hydropathy plot which
 was used to predict the hydrophobic and hydrophilic regions of the full length
 polypeptide.
 10

The HGPRBMY2 sequence begins with a methionine in a DNA sequence context
 consistent with a translation initiation site. An alignment between the HGPRBMY2
 polypeptide with neuropeptide, orexin and galanin receptor sequences is shown in Figure
 9 (par2_human, Genbank Accession No. gil18560788, SEQ ID NO:36; par3_human,
 15 Genbank Accession No. NP_004092, SEQ ID NO:37; thrombin_Xeno, Genbank
 Accession No. gil2134162, SEQ ID NO:38; thrombin_human, Genbank Accession No.
 NP_001983, SEQ ID NO:39; par4_human, Genbank Accession No. NP_003941, SEQ
 ID NO:40; and p2y9_human, Genbank Accession No. gil17426979, SEQ ID NO:41).
 Although the overall amino acid sequence identity between these molecules is low, there
 20 are numerous residues which are conserved across all of the GPCRs in the alignment
 suggesting a functional importance for that residue.

The HGPRBMY1 amino acid sequences of the invention include the amino acid
 sequence shown in Figure 2 (SEQ ID NO:2). The cDNA sequence (SEQ ID NO:1)
 25 described in Section 5.1 encodes the amino acid sequence of HGPRBMY1 (359 amino
 acids; SEQ ID NO:2). The extracellular domains ("ECD") of HGPRBMY1 extend from
 about amino acid residues 1 to about 27, about 85 to about 88, about 161 to about 186,
 and about 259 to about 276 of SEQ ID NO:2; the transmembrane domains ("TM") of
 HGPRBMY1 extend from about amino acid residues 28 to about 49, about 60 to about
 30 84, about 89 to about 105, about 139 to about 160, about 187 to about 200, about 235 to
 about 258, and about 277 to about 297 of SEQ ID NO:2; and the cytoplasmic domains
 ("CD") of HGPRBMY1 extend from about amino acid residue 50 to about 59, about 106
 to about 138, about 201 to about 234, and about 298 to about 359 of SEQ ID NO:2.

Figure 3 depicts the putative transmembrane regions of the HGPRBMY1
 35 polypeptide as shaded areas of the sequence, and also presents a hydropathy plot which
 was used to predict the hydrophobic and hydrophilic regions of the full length
 polypeptide.

The HGPRBMY1 sequence begins with a methionine in a DNA sequence context
5 consistent with a translation initiation site. An alignment between the HGPRBMY1
polypeptide with thrombin receptor, protease activated receptor (par) and P2Y9-like
receptor sequences is shown in Figure 4 (OX2R_HUMAN, Genbank Accession No.
gil17978555, SEQ ID NO:42; OX2R_RAT, Genbank Accession No. gil6981020, SEQ
ID NO:43;. NY4R_MOUSE, Genbank Accession No. gil1587693, SEQ ID NO:44;
10 NY4R_RAT, Genbank Accession No. gil2494992, SEQ ID NO:45; NY6R_RABBIT,
Genbank Accession No. gil3024242, SEQ ID NO:46; Q9WVD0, Genbank Accession No.
gil5410446, SEQ ID NO:47; O57463, Genbank Accession No. gil2739141, SEQ ID
NO:48; NY2R_HUMAN, Genbank Accession No. NP_000901, SEQ ID NO:49;
15 Q9Y5X5, Genbank Accession No. gil4530469, SEQ ID NO:50; and GALR_MOUSE,
Genbank Accession No. gil3023827, SEQ ID NO:51. Although the overall amino acid
sequence identity between these molecules is low, there are numerous residues which are
conserved across all of the GPCRs in the alignment suggesting a functional importance
for that residue.

20 Peptides and polypeptides of HGPRBMY1 or HGPRBMY2 or mutants thereof
can also be chemically synthesized (*e.g.*, see Creighton, 1983, *Proteins: Structures and
Molecular Principles*, W. H. Freeman & Co., N.Y.). In addition, polypeptides and
peptides of the invention may be produced by recombinant DNA technology using
25 techniques well known in the art for expressing nucleic acid containing HGPRBMY1 or
HGPRBMY2 gene sequences and/or coding sequences. Such methods can be used to
construct expression vectors containing various HGPRBMY1 or HGPRBMY2 nucleic
acid sequences, including those described in Section 5.1, and appropriate transcriptional
and translational control signals.

30 These constructs can be designed to encode and express polypeptides or peptides
corresponding to one or more functional domains of the HGPRBMY1 or HGPRBMY2
(*e.g.*, an ECD, a TM and/or a CD) in any order, truncated or deleted HGPRBMY1 or
HGPRBMY2 (*e.g.*, HGPRBMY1 or HGPRBMY2 in which one or more TM and/or CD
are deleted) as well as fusion polypeptides in which the HGPRBMY1 or HGPRBMY2
35 or truncation/deletion mutant of HGPRBMY1 or HGPRBMY2 is fused to an unrelated
polypeptide (*i.e.*, linked to a heterologous carrier polypeptide) and can be designed on the

basis of the HGPRBMY1 or HGPRBMY2 nucleic acid and HGPRBMY1 or
5 HGPRBMY2 amino acid sequences disclosed in this Section and in Section 5.1, above.

The HGPRBMY1 or HGPRBMY2 polypeptide or peptide may be a soluble derivative, *e.g.*, HGPRBMY1 or HGPRBMY2 domains corresponding to one or more of the CD or ECD (*e.g.*, the four ECD constructed in frame and in tandem without linkers, or likewise the four CD in tandem, or any combination of soluble domains of the
10 polypeptide of the invention); one or more of the ECD or CD linked via a hydrophillic peptide linker sequence and/or a flexible linker sequence (*e.g.*, such as GGSGG); or a truncated or deleted HGPRBMY1 or HGPRBMY2 in which the TM are deleted, the TM and CD are deleted or the TM and ECD are deleted, wherein the peptide or polypeptide
15 can be recovered from the culture, *i.e.*, from the host cell in cases where the HGPRBMY1 or HGPRBMY2 peptide or polypeptide is not secreted, and from the culture media in cases where the HGPRBMY1 or HGPRBMY2 peptide or polypeptide is secreted by the cells. In a preferred embodiment, these polypeptides are soluble in normal physiological conditions.

20 Fusion polypeptides comprising HGPRBMY1 or HGPRBMY2 polypeptide or peptide sequences fused to heterologous sequences can include, but are not limited to, epitope tagged polypeptides or peptides, *e.g.*, GST fusions, Myc-tag, hemagglutinin-tag, histidine-tag, FLAG-tag, etc.; Ig-Fc fusions which stabilize the HGPRBMY1 or
25 HGPRBMY2 polypeptide or peptide and prolong half-life *in vivo*; or fusions to any amino acid sequence that allows the fusion polypeptide to be anchored to the cell membrane, allowing the HGPRBMY1 or HGPRBMY2 domain to be exhibited on the cell surface. The fusion polypeptide can also be constructed with a protease cleavage site between the HGPRBMY1 or HGPRBMY2 and the heterologous sequences in order to
30 allow release from the foreign sequences, *e.g.*, thrombin site or factor Xa.

The polypeptides or peptides of the invention can also be conjugated or fused to a compound, such as an enzyme, fluorescent polypeptide, or luminescent polypeptide which provide a marker function. Examples of suitable marker compounds include
35 horseradish peroxidase, alkaline phosphatase, β -galactosidase, acetylcholinesterase, streptavidin/biotin, avidin/biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin, luminol, luciferase, luciferin, aequorin, ^{125}I , ^{131}I , ^{35}S or ^3H .

Further, a polypeptide or peptide of the invention may be conjugated to a
5 therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A
cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples
include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin,
etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin,
10 dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-
dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and
puromycin and analogs or homologues thereof. Therapeutic agents include, but are not
limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine,
cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa
15 chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide,
busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine
platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin)
and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin,
mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and
20 vinblastine).

In addition, a fusion polypeptide or peptide of the invention may be a conjugate
or fusion with a drug moiety, which is not to be construed as limited to classical chemical
therapeutic agents. For example, the drug moiety may be a polypeptide or polypeptide
25 possessing a desired biological activity. Such polypeptides may include, for example, a
toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide
such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet
derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-
angiogenic agent, *e.g.*, angiostatin or endostatin; or, biological response modifiers such
30 as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-4
("IL-4"), interleukin-6 ("IL-6"), interleukin-7 ("IL-7"), granulocyte macrophage colony
stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"),
interleukin-10 ("IL-10"), interleukin-12 ("IL-12"), interleukin-17 ("IL-15"),
interleukin-17 ("IL-17"), interferon- γ ("IFN- γ "), interferon- α ("IFN- α "), or other immune
35 factors or growth factors.

Further, HGPRBMY1 or HGPRBMY2 polypeptides of other species are
encompassed by the invention. In fact, any HGPRBMY1 or HGPRBMY2 polypeptide

encoded by the HGPRBMY1 or HGPRBMY2 nucleic acid sequences described in
5 Section 5.1, above, are within the scope of the invention.

In another embodiment, polypeptides that are functionally equivalent to the
HGPRBMY1 encoded by the nucleic acid sequences described in Section 5.1, as judged
by any of a number of criteria, including but not limited to the ability to bind agonist or
antagonist, the binding affinity for agonist or antagonist, the resulting biological effect
10 of agonist or antagonist binding, *e.g.*, signal transduction, a change in cellular metabolism
(*e.g.*, ion flux) or change in phenotype when the HGPRBMY1 equivalent is present in
an appropriate cell type (such as the amelioration, prevention or delay of an immune
disorder such as rheumatoid arthritis, leukemia or an immunodeficiency); by its ability
15 to bind or compete with antibodies to HGPRBMY1 receptors; or by its ability to elicit
antibodies that immunospecifically bind to the HGPRBMY1 receptor; etc.

The invention also encompasses polypeptides that are functionally equivalent to
the HGPRBMY2 encoded by the nucleic acid sequences described in Section 5.1, as
judged by any of a number of criteria, including but not limited to the ability to bind an
20 antibody, an agonist or an antagonist, the binding affinity for agonist or antagonist, the
resulting biological effect of agonist or antagonist binding, *e.g.*, signal transduction, a
change in cellular metabolism (*e.g.*, ion flux, tyrosine phosphorylation) or change in
phenotype when the HGPRBMY2 equivalent is present in an appropriate cell type (such
25 as the amelioration, prevention or delay of congestive heart failure); by its ability to bind
or compete with antibodies to HGPRBMY2 receptors; or by its ability to elicit antibodies
that immunospecifically bind to the HGPRBMY2 receptor; etc.

Such functionally equivalent HGPRBMY1 polypeptides include but are not
limited to additions or substitutions of amino acid residues within the amino acid
30 sequence encoded by the HGPRBMY1 nucleic acid sequences described, above, in
Section 5.1, but which result in a silent change, thus producing a functionally equivalent
gene product. Amino acid substitutions may be made on the basis of similarity in polarity,
charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the
residues involved. For example, nonpolar (hydrophobic) amino acids include alanine,
35 leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar
neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and
glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine;

and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

5 Regional charge in the polypeptide can be determined analytically with computer programs, for example as shown in Figure 3, which depicts a hydropathy plot of the polypeptide sequence of Figure 2.

Such functionally equivalent HGPRBMY2 polypeptides include but are not limited to additions or substitutions of amino acid residues within the amino acid
10 sequence encoded by the HGPRBMY2 nucleic acid sequences described, above, in Section 5.1, but which result in a silent change, thus producing a functionally equivalent gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the
15 residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.
20 Regional charges in the polypeptide can be determined analytically with computer programs, for example as shown in Figure 8, which depicts a hydropathy plot of the polypeptide sequence of Figure 7.

While random mutations can be made to HGPRBMY1 or HGPRBMY2 DNA and
25 the resulting mutant HGPRBMY1 or HGPRBMY2 tested for activity, site-directed mutations of the HGPRBMY1 or HGPRBMY2 coding sequence can be engineered using site-directed mutagenesis techniques known to those skilled in the art to generate a mutant HGPRBMY1 or HGPRBMY2 with modulated function, *e.g.*, higher binding affinity for agonist or antagonist, and/or changed signaling capacity, *e.g.*, lower binding
30 affinity for agonist or antagonist.

For example, the alignment of HGPRBMY1 and orexin is shown in Figure 4 in which identical amino acid residues are indicated by a black background. Mutant HGPRBMY1 can be engineered so that regions of identity (indicated by black
background in Figure 4) are maintained, whereas the variable residues (white background
35 in Figure 4) are altered, *e.g.*, by deletion or insertion of an amino acid residue(s) or by substitution of one or more different amino acid residues. Conservative alterations at the variable positions can be engineered in order to produce a mutant HGPRBMY1 that

retains function; *e.g.*, agonist or antagonist binding affinity or signal transduction
5 capability or both. Non-conservative changes can be engineered at these variable
positions to alter function, *e.g.*, agonist or antagonist binding affinity or signal
transduction capability, or both.

For example, the alignment of HGPRBMY2 and orexin is shown in Figure 9 in
which identical amino acid residues are indicated by a black background. Mutant
10 HGPRBMY2 can be engineered so that regions of identity (indicated by black
background in Figure 9) are maintained, whereas the variable residues (white background
in Figure 9) are altered, *e.g.*, by deletion or insertion of an amino acid residue(s) or by
substitution of one or more different amino acid residues. Conservative alterations at the
15 variable positions can be engineered in order to produce a mutant HGPRBMY2 that
retains function; *e.g.*, agonist or antagonist binding affinity or signal transduction
capability or both. Non-conservative changes can be engineered at these variable
positions to alter function, *e.g.*, agonist or antagonist binding affinity or signal
transduction capability, or both.

20 In addition, mutation by deletion or non-conservative alteration of the conserved
regions can be engineered where modulation of function is desired (*i.e.*, identical amino
acids indicated by stars in Figure 4 or Figure 9). For example, deletion or
non-conservative alterations (substitutions or insertions) of the agonist binding domain,
25 can be engineered to produce a mutant HGPRBMY1 or HGPRBMY2 that binds agonist
or antagonist but is signaling-incompetent. Non-conservative alterations to the residues
with a black background in the ECD shown in Figure 4 or Figure 9 can be engineered to
produce mutant HGPRBMY1 or HGPRBMY2 with altered binding affinity for agonist
or antagonist.

30 Other mutations to the HGPRBMY1 or HGPRBMY2 coding sequence can be
made to generate HGPRBMY1 or HGPRBMY2 that are better suited for expression in
host cells, *e.g.*, reduced toxicity, increased solubility, scale up, etc. in host cells. For
example, cysteine residues can be deleted or substituted with another amino acid in order
to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated
35 to achieve, for example, expression of a homogeneous product that is more easily
recovered and purified from yeast hosts which are known to hyperglycosylate N-linked
sites. To this end, a variety of amino acid substitutions at one or both of the first or third

amino acid positions of any one or more of the glycosylation recognition sequences
5 which occur in an ECD (N-X-S or N-X-T), and/or an amino acid deletion at the second
position of any one or more such recognition sequences in the ECD will prevent
glycosylation of the HGPRBMY1 or HGPRBMY2 at the modified tripeptide sequence.
(See, *e.g.*, Miyajima et al., 1986, EMBO J. 5(6):1193-1197). In addition, the nucleic acid
10 construct can be designed to be polycistronic with alternative splice sites in order to
increase production of polypeptides or peptides of the invention per cell, thus increasing
yield.

The expression systems also encompass engineered host cells that express the
HGPRBMY1 or HGPRBMY2 or functional equivalents *in situ*, *i.e.*, anchored in the cell
15 membrane. Purification or enrichment of the HGPRBMY1 or HGPRBMY2 from such
expression systems can be accomplished using appropriate detergents and lipid micelles
and methods well known to those skilled in the art. However, such engineered host cells
themselves may be used in situations where it is important not only to retain the structural
and functional characteristics of the HGPRBMY1 or HGPRBMY2, but to assess
20 biological activity, *e.g.*, in drug screening assays.

The expression systems that may be used for purposes of the invention include
but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*)
transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA
25 expression vectors containing HGPRBMY1 or HGPRBMY2 nucleic acid sequences;
yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression
vectors containing the HGPRBMY1 or HGPRBMY2 nucleic acid sequences; insect cell
systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing
the HGPRBMY1 or HGPRBMY2 sequences; plant cell systems infected with
30 recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco
mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*,
Ti plasmid) containing HGPRBMY1 or HGPRBMY2 nucleic acid sequences; or
mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3) harboring recombinant
expression constructs containing promoters derived from the genome of mammalian cells
35 (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late
promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously
5 selected depending upon the use intended for the HGPRBMY1 or HGPRBMY2 gene
product being expressed. For example, when a large quantity of such a polypeptide is to
be produced, for the generation of pharmaceutical compositions of HGPRBMY1 or
HGPRBMY2 polypeptide or for raising antibodies to the HGPRBMY1 or HGPRBMY2
10 polypeptide, for example, vectors which direct the expression of high levels of fusion
polypeptide products that are readily purified may be desirable. For example, pGEX
vectors may also be used to express foreign polypeptides as fusion polypeptides with
glutathione S-transferase (GST). The pGEX vectors are designed to include thrombin or
factor Xa protease cleavage sites so that the cloned target gene product can be released
15 from the GST moiety.

In an insect system, Autographa californica nuclear polyhidrosis virus (AcNPV)
is used as a vector to express foreign genes. These recombinant viruses are then used to
infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (*e.g.*, see
Smith et al., 1983, J. Virol. 46: 584; Smith, U.S. Pat. No. 4,215,051).

20 In mammalian host cells, a number of viral-based expression systems may be
utilized. In adenovirus, the HGPRBMY1 or HGPRBMY2 nucleic acid sequence of
interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*,
the late promoter and tripartite leader sequence. This chimeric gene may then be inserted
25 in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a
non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a
recombinant virus that is viable and capable of expressing the HGPRBMY1 or
HGPRBMY2 gene product in infected hosts (*e.g.*, See Logan & Shenk, 1984, Proc. Natl.
Acad. Sci. USA 81:3655-3659).

30 In cases where an entire HGPRBMY1 or HGPRBMY2 gene or cDNA, including
its own initiation codon and adjacent sequences, is inserted into the appropriate
expression vector, no additional translational control signals may be needed. However,
in cases where only a portion of the HGPRBMY1 or HGPRBMY2 coding sequence is
inserted, exogenous translational control signals, including, perhaps, the ATG initiation
35 codon, must be provided. Furthermore, the initiation codon must be correctly oriented in
the reading frame of the desired coding sequence to ensure translation of the insert in the
correct reading frame. These exogenous translational control signals and initiation codons

can be of a variety of origins, both natural and synthetic. Intronic sequences and
5 polyadenylation signals can also be included to increase the efficiency of expression.

In addition, a host cell strain may be chosen which modulates the expression of
the inserted sequences, or modifies and processes the gene product in the specific fashion
desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of
10 polypeptide products may be important for the function of the polypeptide. Different host
cells have characteristic and specific mechanisms for the post-translational processing
and modification of polypeptides and gene products. Appropriate cell lines or host
systems can be chosen to ensure the correct modification and processing of the foreign
polypeptide expressed. To this end, eukaryotic host cells which possess the cellular
15 machinery for proper processing of the primary transcript, glycosylation, and
phosphorylation of the gene product may be used. Such mammalian host cells include but
are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in
particular, bone marrow cell lines such as lymphocyte lineage (for example, monocyte,
B-cell or T-cell, such as K562, WEHI 7.1 or WEHI-3 cell lines) or erythrocyte lineage
20 cell lines.

For long-term, high-yield production of recombinant polypeptides, stable
expression is preferred. For example, cell lines which stably express the HGPRBMY1
or HGPRBMY2 sequences described above may be engineered. Rather than using
25 expression vectors which contain viral origins of replication, host cells can be
transformed with DNA controlled by appropriate expression control elements (*e.g.*,
promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and
a selectable marker. Following the introduction of the foreign DNA, engineered cells may
be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective
30 media. The selectable marker in the recombinant plasmid confers resistance to the
selection and allows cells to stably integrate the plasmid into their chromosomes and
grow to form foci which in turn can be cloned and expanded into cell lines. This method
may advantageously be used to engineer cell lines which express the HGPRBMY1 or
HGPRBMY2 gene product. Such engineered cell lines may be particularly useful in
35 screening and evaluation of compounds that affect the endogenous activity of the
HGPRBMY1 or HGPRBMY2 gene product.

A number of selection systems may be used, including but not limited to the
5 herpes simplex virus thymidine kinase (tk) (Wigler, *et al.*, 1977, Cell 11:223),
hypoxanthine-guanine phosphoribosyltransferase (hgp^rt) (Szybalska & Szybalski, 1962,
Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (ap^rt)
(Lowy, *et al.*, 1980, Cell 22:817) genes can be employed in tk⁻, hgp^rt⁻ or ap^rt⁻ cells,
10 respectively. Also, antimetabolite resistance can be used as the basis of selection for the
following genes: Dihydrofolate Reductase (DHFR), which confers resistance to
methotrexate (Wigler, *et al.*, 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, *et al.*, 1981,
Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid
(Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neomycin, which confers
15 resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, J. Mol. Biol.
150:1); and hyg^r, which confers resistance to hygromycin (Santerre, *et al.*, 1984, Gene
30:147).

The polypeptides of the invention can, for example, include modifications that
can increase such attributes as stability, half-life, ability to enter cells and aid in
20 administration, *e.g.*, *in vivo* administration of the polypeptides of the invention. For
example, polypeptides of the invention can comprise a polypeptide transduction domain
of the HIV TAT polypeptide as described in Schwarze, *et al.* (1999 *Science* 285:1569-
1572), thereby facilitating delivery of polypeptides of the invention into cells.

25 Alternatively, any fusion polypeptide may be readily purified by utilizing an
antibody specific for the fusion polypeptide being expressed. For example, a system
described by Janknecht *et al.* allows for the ready purification of non-denatured fusion
polypeptides expressed in human cell lines (Janknecht, *et al.*, 1991, Proc. Natl. Acad. Sci.
USA 88: 8972-8976). In this system, the gene of interest is subcloned into a vaccinia
30 recombination plasmid such that the gene's open reading frame is translationally fused
to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected
with recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose columns
and histidine-tagged polypeptides are selectively eluted with imidazole-containing
35 buffers.

The HGPRBMY1 or HGPRBMY2 gene products can also be expressed in
transgenic animals. Animals of any species, including, but not limited to, mice, rats,
rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, *e.g.*, baboons,

monkeys, and chimpanzees may be used to generate HGPRBMY1 or HGPRBMY2
5 transgenic animals.

Any technique known in the art may be used to introduce the HGPRBMY1 or
HGPRBMY2 transgene into animals to produce the founder lines of transgenic animals.
Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P. C.
and Wagner, T. E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into
10 germ lines (Van der Putten et al., 1985, Proc. Natl. Acad. Sci., USA 82:6148-6152); gene
targeting in embryonic stem cells (Thompson et al., 1989, Cell 56:313-321);
electroporation of embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814); and sperm-mediated
gene transfer (Lavitrano et al., 1989, Cell 57:717-723); etc. For a review of such
15 techniques, see Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115:171-229, which
is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals that carry the HGPRBMY1
or HGPRBMY2 transgene in all their cells, as well as animals which carry the transgene
in some, but not all their cells, *i.e.*, mosaic animals. The transgene may be integrated as
20 a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems.
The transgene may also be selectively introduced into and activated in a particular cell
type by following, for example, the teaching of Lasko et al. (Lasko, M. et al., 1992, Proc.
Natl. Acad. Sci. USA 89: 6232-6236). The regulatory sequences required for such a
25 cell-type specific activation will depend upon the particular cell type of interest, and will
be apparent to those of skill in the art. When it is desired that the HGPRBMY1 or
HGPRBMY2 gene transgene be integrated into the chromosomal site of the endogenous
HGPRBMY1 or HGPRBMY2 gene, gene targeting is preferred. Briefly, when such a
technique is to be utilized, vectors containing some nucleic acid sequences homologous
30 to the endogenous HGPRBMY1 or HGPRBMY2 gene are designed for the purpose of
integrating, via homologous recombination with chromosomal sequences, into and
disrupting the function of the nucleic acid sequence of the endogenous HGPRBMY1 or
HGPRBMY2 gene. The transgene may also be selectively introduced into a particular cell
type, thus inactivating the endogenous HGPRBMY1 or HGPRBMY2 gene in only that
35 cell type, by following, for example, the teaching of Gu et al. (Gu, et al., 1994, Science
265: 103-106). The regulatory sequences required for such a cell-type specific

inactivation will depend upon the particular cell type of interest, and will be apparent to
5 those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant
HGPRBMY1 or HGPRBMY2 gene may be assayed utilizing standard techniques. Initial
screening may be accomplished by Southern blot analysis or PCR techniques to analyze
10 animal tissues to assay whether integration of the transgene has taken place. The level of
mRNA expression of the transgene in the tissues of the transgenic animals may also be
assessed using techniques which include but are not limited to Northern blot analysis of
tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR.
Samples of HGPRBMY1 or HGPRBMY2 gene-expressing tissue, may also be evaluated
15 immunocytochemically using antibodies specific for the HGPRBMY1 or HGPRBMY2
transgene product.

5.3. Antibodies to HGPRBMY1

Antibodies that specifically recognize one or more epitopes of HGPRBMY1 or
20 HGPRBMY2, or epitopes of conserved variants of HGPRBMY1 or HGPRBMY2
polypeptides or peptides, are also encompassed by the invention. Such antibodies include
but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized
or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments,
25 fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and
epitope-binding fragments of any of the above.

The antibodies of the invention may be used, for example, in the detection of the
HGPRBMY1 in a biological sample and may, therefore, be utilized as part of a diagnostic
or prognostic technique whereby patients may be tested for abnormal amounts of
30 HGPRBMY1. Such antibodies may also be utilized in conjunction with, for example,
compound screening schemes, as described, below, in Section 5.5, for the evaluation of
the effect of test compounds on expression and/or activity of the HGPRBMY1 gene
product. Additionally, such antibodies can be used in conjunction with the gene therapy
techniques described, below, in Section 5.6, to, for example, evaluate the normal and/or
35 engineered HGPRBMY1-expressing cells prior to their introduction into the patient. Such
antibodies may additionally be used as a method for the inhibition of abnormal

HGPRBMY1 activity. Thus, such antibodies may, therefore, be utilized as part of
5 immune disorder treatment methods.

The antibodies of the invention may be used, for example, in the detection of the
HGPRBMY2 in a biological sample and may, therefore, be utilized as part of a diagnostic
or prognostic technique whereby patients may be tested for abnormal amounts of
HGPRBMY2. Such antibodies may also be utilized in conjunction with, for example,
10 compound screening schemes, as described, below, in Section 5.5, for the evaluation of
the effect of test compounds on expression and/or activity of the HGPRBMY2 gene
product. Additionally, such antibodies can be used in conjunction with the gene therapy
techniques described, below, in Section 5.6, to, for example, evaluate the normal and/or
15 engineered HGPRBMY2-expressing cells prior to their introduction into the patient. Such
antibodies may additionally be used as a method for the inhibition of abnormal
HGPRBMY2 activity. Thus, such antibodies may, therefore, be utilized as part of heart
disorder treatment methods.

In a particular embodiment, HGPRBMY1 expression can be utilized as a marker
20 (*e.g.*, an *in situ* marker) for specific tissues (*e.g.*, bone marrow, spleen or thymus) and/or
cells (*e.g.*, lymphocytes) in which HGPRBMY1 is expressed.

In a particular embodiment, HGPRBMY2 expression can be utilized as a marker
(*e.g.*, an *in situ* marker) for specific tissues (*e.g.*, heart, brain, etc.) and/or cells (*e.g.*, cells
25 shown in Figures 10 and 16) in which HGPRBMY2 is expressed.

An isolated polypeptide or peptide of the invention can be used as an immunogen
to generate antibodies using standard techniques for polyclonal and monoclonal antibody
preparation. The full-length polypeptide or a functional domain of the polypeptide, either
native or denatured, can be used or, alternatively, the invention provides antigenic
30 polypeptides or peptides for use as immunogens. The antigenic peptide of a polypeptide
of the invention comprises at least 8 (preferably 10, 15, 20, or 30) amino acid residues
of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a variant thereof, and
features an epitope of the polypeptide such that an antibody raised against the peptide
forms a specific immune complex with the polypeptide, and alternatively with a native
35 polypeptide.

Preferred epitopes encompassed by the antigenic peptide are regions that are
located on the surface of the polypeptide, *e.g.*, hydrophilic regions, for example, as shown

in hydrophilic regions in Figure 3 or Figure 8. In certain embodiments, the nucleic acid
5 molecules of the invention are present as part of nucleic acid molecules comprising
nucleic acid sequences that contain or encode heterologous (*e.g.*, vector, expression
vector, or fusion polypeptide) sequences. These nucleotides can then be used to express
polypeptides which can be used as immunogens to generate an immune response, or more
10 particularly, to generate polyclonal or monoclonal antibodies specific to the expressed
polypeptide.

An immunogen typically is used to prepare antibodies by immunizing a suitable
subject, (*e.g.*, rabbit, goat, mouse or other mammal). An appropriate immunogenic
preparation can contain, for example, recombinantly expressed or chemically synthesized
15 polypeptide. The preparation can further include an adjuvant, such as Freund's complete
or incomplete adjuvant, or similar immunostimulatory agent.

Accordingly, another aspect of the invention pertains to antibodies directed
against a polypeptide of the invention. The term "antibody" as used herein refers to
immunoglobulin molecules and immunologically active portions of immunoglobulin
20 molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds
an antigen, such as a polypeptide of the invention, *e.g.*, an epitope of a polypeptide of the
invention. A molecule which specifically binds to a given polypeptide of the invention
is a molecule which binds the polypeptide, but does not substantially bind other
25 molecules in a sample, *e.g.*, a biological sample, which naturally contains the
polypeptide. Examples of immunologically active portions of immunoglobulin molecules
include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with
an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies.
The term "monoclonal antibody" or "monoclonal antibody composition", as used herein,
30 refers to a population of antibody molecules that contain only one species of an antigen
binding site capable of immunoreacting with a particular epitope.

Polyclonal antibodies can be prepared by immunizing a suitable subject with a
polypeptide of the invention as an immunogen. Preferred polyclonal antibody
compositions are ones that have been selected for antibodies directed against a
35 polypeptide or polypeptides of the invention. Particularly preferred polyclonal antibody
preparations are ones that contain only antibodies directed against a polypeptide or
polypeptides of the invention. Particularly preferred immunogen compositions are those

that contain no other human polypeptides such as, for example, immunogen compositions
5 made using a non-human host cell for recombinant expression of a polypeptide of the
invention. In such a manner, the only human epitope or epitopes recognized by the
resulting antibody compositions raised against this immunogen will be present as part of
a polypeptide or polypeptides of the invention.

10 The antibody titer in the immunized subject can be monitored over time by
standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using
immobilized polypeptide. If desired, the antibody molecules can be isolated from the
mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as
protein A chromatography to obtain the IgG fraction. Alternatively, antibodies specific
15 for a polypeptide or peptide of the invention can be selected for (*e.g.*, partially purified)
or purified by, *e.g.*, affinity chromatography. For example, a recombinantly expressed and
purified (or partially purified) polypeptide of the invention is produced as described
herein, and covalently or non-covalently coupled to a solid support such as, for example,
a chromatography column. The column can then be used to affinity purify antibodies
20 specific for the polypeptides of the invention from a sample containing antibodies
directed against a large number of different epitopes, thereby generating a substantially
purified antibody composition, *i.e.*, one that is substantially free of contaminating
antibodies. By a substantially purified antibody composition is meant, in this context, that
25 the antibody sample contains at most only 30% (by dry weight) of contaminating
antibodies directed against epitopes other than those on the desired polypeptide or
polypeptide of the invention, and preferably at most 20%, yet more preferably at most
10%, and most preferably at most 5% (by dry weight) of the sample is contaminating
antibodies. A purified antibody composition means that at least 99% of the antibodies in
30 the composition are directed against the desired polypeptide or peptide of the invention.

At an appropriate time after immunization, *e.g.*, when the specific antibody titers
are highest, antibody-producing cells can be obtained from the subject and used to
prepare monoclonal antibodies by standard techniques, such as the hybridoma technique
originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B
35 cell hybridoma technique (Kozbor et al. (1983) *Immunol. Today* 4:72), the
EBV-hybridoma technique (Cole et al. (1985), *Monoclonal Antibodies and Cancer
Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for

producing hybridomas is well known (*see generally Current Protocols in Immunology*
5 (1994) Coligan et al. (eds.) John Wiley & Sons, Inc., New York, NY). Hybridoma cells
producing a monoclonal antibody of the invention are detected by screening the
hybridoma culture supernatants for antibodies that bind the polypeptide of interest, *e.g.*,
using a standard ELISA assay.

10 Alternative to preparing monoclonal antibody-secreting hybridomas, a
monoclonal antibody directed against a polypeptide of the invention can be identified and
isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an
antibody phage display library) with the polypeptide of interest. Kits for generating and
screening phage display libraries are commercially available (*e.g.*, the Pharmacia
15 *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene
SurfZAP Phage Display Kit, Catalog No. 240612). Additionally, examples of methods
and reagents particularly amenable for use in generating and screening antibody display
library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No.
WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791;
20 PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT
Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication
No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992)
Hum. Antibod. Hybridomas 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths
25 et al. (1993) *EMBO J.* 12:725-734.

Additionally, recombinant antibodies, such as chimeric and humanized
monoclonal antibodies, comprising both human and non-human portions, which can be
made using standard recombinant DNA techniques, are within the scope of the invention.
A chimeric antibody is a molecule in which different portions are derived from different
30 animal species, such as those having a variable region derived from a murine mAb and
a human immunoglobulin constant region. (See, *e.g.*, Cabilly et al., U.S. Patent No.
4,816,567; and Boss et al., U.S. Patent No. 4,816,397, which are incorporated herein by
reference in their entirety.) Humanized antibodies are antibody molecules from non-
human species having one or more complementarily determining regions (CDRs) from
35 the non-human species and a framework region from a human immunoglobulin molecule
(See, *e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference
in its entirety.) Such chimeric and humanized monoclonal antibodies can be produced by

recombinant DNA techniques known in the art, for example using methods described in
5 PCT Publication No. WO 87/02671; European Patent Application 184,187; European
Patent Application 171,496; European Patent Application 173,494; PCT Publication No.
WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better
et al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *Proc. Natl. Acad. Sci. USA*
84:3439-3443; Liu et al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987) *Proc. Natl.*
10 *Acad. Sci. USA* 84:214-218; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et
al. (1985) *Nature* 314:446-449; and Shaw et al. (1988) *J. Natl. Cancer Inst.*
80:1553-1559; Morrison (1985) *Science* 229:1202-1207; Oi et al. (1986) *Bio/Techniques*
4:214; U.S. Patent 5,225,539; Jones et al. (1986) *Nature* 321:552-525; Verhoeyan et al.
15 (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment
of human patients. Such antibodies can be produced, for example, using transgenic mice
which are incapable of expressing endogenous immunoglobulin heavy and light chains
genes, but which can express human heavy and light chain genes. The transgenic mice
20 are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a
polypeptide of the invention. Monoclonal antibodies directed against the antigen can be
obtained using conventional hybridoma technology. The human immunoglobulin
transgenes harbored by the transgenic mice rearrange during B cell differentiation, and
subsequently undergo class switching and somatic mutation. Thus, using such a
25 technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies.
For an overview of this technology for producing human antibodies, see Lonberg and
Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology
for producing human antibodies and human monoclonal antibodies and protocols for
30 producing such antibodies, *see, e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S.
Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition,
companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human
antibodies directed against a selected antigen using technology similar to that described
above.

35 Completely human antibodies which recognize a selected epitope can be
generated using a technique referred to as "guided selection." In this approach a selected
non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection

of a completely human antibody recognizing the same epitope (Jespers et al. (1994)

5 *Bio/technology* 12:899-903).

An antibody directed against a polypeptide of the invention (*e.g.*, monoclonal antibody) can be used to isolate the polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, such an antibody can be used to detect the polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. The antibodies can also be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

In addition, the HGPRBMY1 or HGPRBMY2 gene sequences and gene products, including polypeptides, peptides, fusion polypeptides or peptides, and antibodies directed against said gene products and peptides, have applications for purposes independent of the role of the gene products. For example, HGPRBMY1 or HGPRBMY2 gene products, including polypeptides or peptides, as well as specific antibodies thereto, can be used for construction of fusion polypeptides to facilitate recovery, detection, or localization of another polypeptide of interest. In addition, HGPRBMY1 or HGPRBMY2 genes and gene products can be used for genetic mapping. Finally, HGPRBMY1 or HGPRBMY2 nucleic acids and gene products have generic uses, such as supplemental sources of nucleic acids, polypeptides and amino acids for food additives or cosmetic products.

Further, an antibody of the invention (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion.

A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples
5 include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin,
etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin,
dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-
dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and
10 puromycin and analogs or homologues thereof. Therapeutic agents include, but are not
limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine,
cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa
chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide,
busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine
15 platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin)
and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin,
mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and
vinblastine).

20 In addition, polypeptides, agonists or antagonists which bind a polypeptide of the
invention can also be conjugated to the foregoing, thereby targeting a toxin to cells
expressing HGPRBMY1 or HGPRBMY2.

The conjugates of the invention can be used for modifying a given biological
response, the drug moiety is not to be construed as limited to classical chemical
25 therapeutic agents. For example, the drug moiety may be a polypeptide or peptide
possessing a desired biological activity. Such polypeptides may include, for example, a
toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide
such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet
derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-
30 angiogenic agent, *e.g.*, angiostatin or endostatin; or, biological response modifiers such
as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-4
("IL-4"), interleukin-6 ("IL-6"), interleukin-7 ("IL-7"), granulocyte macrophage colony
stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"),
interleukin-10 ("IL-10"), interleukin-12 ("IL-12"), interleukin-17 ("IL-15"),
35 interleukin-17 ("IL-17"), interferon- γ ("IFN- γ "), interferon- α ("IFN- α "), or other immune
factors or growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known,
5 see, *e.g.*, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer
Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-
56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in
Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker,
10 Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A
Review", in *Monoclonal Antibodies: Biological And Clinical Applications*, Pinchera et
al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The
Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal
Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16
15 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties
Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982).

An antibody with or without a therapeutic moiety conjugated to it can be used as
a therapeutic that is administered alone or in combination with chemotherapeutic agents.

Alternatively, an antibody of the invention can be conjugated to a second
20 antibody to form an "antibody heteroconjugate" as described by Segal in U.S. Patent No.
4,676,980 or alternatively, the antibodies can be conjugated to form an "antibody
heteropolymer" as described in Taylor *et al.*, in U.S. Patent Nos. 5,470,570 and
5,487,890.

25 An antibody with or without a therapeutic moiety conjugated to it can be used as
a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or
cytokine(s).

In yet a further aspect, the invention provides substantially purified antibodies or
fragments thereof, including human or non-human antibodies or fragments thereof, which
30 antibodies or fragments specifically bind to a polypeptide of the invention comprising an
amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a variant thereof. In various
embodiments, the substantially purified antibodies of the invention, or fragments thereof,
can be human, non-human, chimeric and/or humanized antibodies.

35 In another aspect, the invention provides human or non-human antibodies or
fragments thereof, which antibodies or fragments specifically bind to a polypeptide
comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a variant
thereof. Such non-human antibodies can be goat, mouse, sheep, horse, chicken, rabbit,

or rat antibodies. Alternatively, the non-human antibodies of the invention can be
5 chimeric and/or humanized antibodies. In addition, the non-human antibodies of the
invention can be polyclonal antibodies or monoclonal antibodies.

In still a further aspect, the invention provides monoclonal antibodies or
fragments thereof, which antibodies or fragments specifically bind to a polypeptide of the
invention comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a
10 variant thereof. The monoclonal antibodies can be human, humanized, chimeric and/or
non-human antibodies.

The substantially purified antibodies or fragments thereof specifically bind to a
signal peptide, a secreted sequence, an extracellular domain, a transmembrane or a
15 cytoplasmic domain cytoplasmic membrane of a polypeptide of the invention. In a
particularly preferred embodiment, the substantially purified antibodies or fragments
thereof, the non-human antibodies or fragments thereof, and/or the monoclonal antibodies
or fragments thereof, of the invention specifically bind to a secreted sequence, or
alternatively, to an extracellular domain of the amino acid sequence of the invention..

20 Any of the antibodies of the invention can be conjugated to a therapeutic moiety
or to a detectable substance. Non-limiting examples of detectable substances that can be
conjugated to the antibodies of the invention are an enzyme, a prosthetic group, a
fluorescent material, a luminescent material, a bioluminescent material, and a radioactive
25 material.

The invention also provides a kit containing an antibody of the invention
conjugated to a detectable substance, and instructions for use. Still another aspect of the
invention is a pharmaceutical composition comprising an antibody of the invention and
a pharmaceutically acceptable carrier. In preferred embodiments, the pharmaceutical
30 composition contains an antibody of the invention, a therapeutic moiety, and a
pharmaceutically acceptable carrier.

Still another aspect of the invention is a method of making an antibody that
specifically recognizes HGPRBMY1 or HGPRBMY2, the method comprising
immunizing a mammal with a polypeptide. After immunization, a sample is collected
35 from the mammal that contains an antibody that specifically recognizes the immunogen.
Preferably, the polypeptide is recombinantly produced using a non-human host cell.
Optionally, the antibodies can be further purified from the sample using techniques well

known to those of skill in the art. The method can further comprise producing a
5 monoclonal antibody-producing cell from the cells of the mammal. Optionally, antibodies
are collected from the antibody-producing cell.

Alternatively, techniques described for the production of single chain antibodies
(U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc.
Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be
10 adapted to produce single chain antibodies against HGPRBMY1 or HGPRBMY2 gene
products. Single chain antibodies are formed by linking the heavy and light chain
fragments of the Fv region via an amino acid bridge, resulting in a single chain
polypeptide.

15 Antibodies to the HGPRBMY1 can, in turn, be utilized to generate anti-idiotypic
antibodies that "mimic" the HGPRBMY1, using techniques well known to those skilled
in the art (See, *e.g.*, Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff,
1991, J. Immunol. 147(8):2429-2438). For example antibodies which bind to the
HGPRBMY1 ECD and competitively inhibit the binding of agonist or antagonist to the
20 HGPRBMY1 can be used to generate anti-idiotypes that "mimic" the ECD and, therefore,
bind and neutralize agonist or antagonist. Such neutralizing anti-idiotypes or Fab
fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize agonist
or antagonist and prevent immune disorders.

25 Antibodies to the HGPRBMY2 can, in turn, be utilized to generate anti-idiotypic
antibodies that "mimic" the HGPRBMY2, using techniques well known to those skilled
in the art. (See, *e.g.*, Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff,
1991, J. Immunol. 147(8):2429-2438). For example antibodies which bind to the
HGPRBMY2 ECD and competitively inhibit the binding of agonist or antagonist to the
30 HGPRBMY2 can be used to generate anti-idiotypes that "mimic" the ECD and, therefore,
bind and neutralize agonist or antagonist. Such neutralizing anti-idiotypes or Fab
fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize agonist
or antagonist and prevent heart failure or neural disorders.

35 **5.4. Diagnosis of Immune Disorders**

A variety of methods can be employed for the diagnostic and prognostic
5 evaluation of immune disorders and for the identification of subjects having a
predisposition to such disorders.

Immune system disorders occur when the immune response is inappropriate,
excessive, or lacking. Immunodeficiency disorders occur when the immune system fails
10 to fight tumors or invading substances. This causes persistent or recurrent infections,
severe infections by organisms that are normally mild, incomplete recovery from illness
or poor response to treatment, and increased incidence of cancer and other tumors.
Opportunistic infections are widespread infections by microorganisms that are usually
controllable.

15 People are said to be "immunosuppressed" when they experience
immunodeficiency that is caused by medications such as corticosteroids or
immunosuppressant (chemotherapy) medications. This is a desired part of treatment for
disorders such as autoimmune disorders. It is used after organ transplantation to prevent
transplant rejection. Acquired immunodeficiency may be a complication of diseases such
20 as HIV, infection and AIDS (acquired immunodeficiency syndrome), or from
malnutrition.

Various immune disorders include, but are not limited to: congenital
immunodeficiency, Anemia, Antiphospholipid Syndrome (APS), Blue Rubber Bleb
25 Nevus Syndrome, Gout, Hemophilia, Leukemia, Myeloproliferative Disorders, Sickle
Cell Disease, and Thalassemia. Additionally, diseases which affect immune function are
contemplated, for example those that cause immunodeficiency such as AIDS/HIV.

Examples of congenital immunodeficiency disorders of antibody production (B
lymphocyte abnormalities) include hypo-gammaglobulinemia (lack of one or more
30 specific antibodies), which usually causes repeated mild respiratory infections, and
agammaglobulinemia (lack of all or most antibody production), which results in frequent
severe infections and is often fatal. Congenital disorders affecting the T lymphocytes may
cause increased susceptibility to fungi, resulting in repeated Candida (yeast) infections.
Inherited combined immunodeficiency affects both T lymphocytes and B lymphocytes.

35 The following conditions and diseases often result in an immunodeficient state:
ataxia-telangiectasia, DiGeorge syndrome, Chediak-Higashi syndrome, Job syndrome,
leukocyte adhesion defects, panhypogammaglobulinemia, Bruton disease, congenital

agammaglobulinemia, selective deficiency of IgA, combined immunodeficiency disease,
5 Wiscott-Aldrich syndrome, and complement deficiencies.

Suppression of the immune system may be desired in the treatment of certain disorders, or it may be a side effect of some treatments, for example in organ or bone marrow transplantation.

10 Immune deficiency is identified partly by poor response to treatment, delayed or incomplete recovery from illness, the presence of certain types of cancers (such as Kaposi's sarcoma), opportunistic infections (such as widespread *Pneumocystis carinii* infection or recurrent fungal/yeast infections).

Autoimmune disorders occur when the normal control process is disrupted. They
15 may also occur if normal body tissue is altered so that it is no longer recognized as "self." Because autoimmune disorders and allergy are both caused by hypersensitivity reactions, it is believed that a history of allergy indicates increased risk for autoimmune disorders.

Examples of autoimmune (or autoimmune-related) disorders include but are not limited to: Hashimoto's thyroiditis, pernicious anemia, Addison's disease, diabetes
20 mellitus, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, dermatomyositis, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, and Graves disease.

Additional immune disorders include: Giant Lymph Node Hyperplasia,
25 Castleman disease, Small Bowel Nodules, Immunoblastic Lymphadenopathy, Immunoproliferative Small Intestinal Disease, myelodysplasia syndrome 1, Still's syndrome, Lymphangiomyoma, Lymphoma, Abdominal Visceral Lymphoma, Bilaterally Large Multifocal Kidneys, Marek's Disease, Sezary Syndrome, Mycosis Fungoides and Tumor Lysis Syndrome.

30 Organs and tissues commonly affected by autoimmune disorders include blood components such as red blood cells, blood vessels, connective tissues, endocrine glands such as the thyroid or pancreas, muscles, joints, and skin. A person may experience more than one autoimmune disorder at the same time. Some disorders have multiple interrelated causes, one of which is autoimmunity.

35 Leukemias are defined generally as a group of usually fatal diseases of the reticuloendothelial system involving uncontrolled proliferation of white blood cells (leukocytes) such as: chronic myelogenous leukemia (CML), hairy cell leukemia, chronic

lymphocytic leukemia (CLL), acute lymphocytic leukemia, acute nonlymphocytic
5 leukemia (AML), and chronic myelomonocytic leukemia.

Moreover, as the compositions of the invention relate to bone marrow, it is also contemplated that BMPRBMY1 can be targeted for modulation of anemia. Anemias which can be treated by methods of the invention include but are not limited to: anemia of B12 deficiency, anemia of chronic disease, anemia of folate deficiency, drug-induced
10 immune hemolytic anemia, hemolytic anemia, hemolytic anemia due to g6pd deficiency, idiopathic aplastic anemia, idiopathic autoimmune hemolytic anemia, immune hemolytic anemia, iron deficiency anemia, megaloblastic anemia, pernicious anemia, secondary aplastic anemia, and sickle cell anemia.

Other HGPRBMY1 associated disorders can include TNF related disorders (*e.g.*, acute myocarditis, myocardial infarction, congestive heart failure, T cell disorders (*e.g.*, dermatitis, fibrosis)), immunological differentiative and apoptotic disorders (*e.g.*, hyper-proliferative syndromes such as systemic lupus erythematosus (lupus)), and disorders related to angiogenesis (*e.g.*, tumor formation and/or metastasis, cancer). Modulators of
20 HGPRBMY1 expression and/or activity can be used to treat such disorders.

Methods of diagnosing or detecting immune disorders may, for example, utilize reagents such as the HGPRBMY1 nucleic acid sequences described in Section 5.1, and HGPRBMY1 antibodies, as described, in Section 5.3. Specifically, such reagents may be
25 used, for example, for: (1) the detection of the presence of HGPRBMY1 gene mutations, or the detection of either over- or under-expression of HGPRBMY1 mRNA relative to the non-immune related disorder state; (2) the detection of either an over- or an under-abundance of HGPRBMY1 gene product relative to the non-immune related disorder state; and (3) the detection of perturbations or abnormalities in the signal
30 transduction pathway mediated by HGPRBMY1.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific HGPRBMY1 nucleic acid sequence or HGPRBMY1 antibody reagent described herein, which may be conveniently
35 used, *e.g.*, in clinical settings, to diagnose patients exhibiting immune related disorder abnormalities.

For the detection of HGPRBMY1 mutations, any nucleated cell can be used as a starting source for genomic nucleic acid. For the detection of HGPRBMY1 gene

expression or HGPRBMY1 gene products, any cell type or tissue in which the
5 HGPRBMY1 gene is expressed, such as, for example, immune cells, may be utilized.

Nucleic acid-based detection techniques are described, below, in Section 5.4.1.

Peptide detection techniques are described, below, in Section 5.4.2.

10 5.4b. Diagnosis of Cardiovascular Disorders

A variety of methods can be employed for the diagnostic and prognostic
evaluation of HGPRBMY2-related cardiovascular disorders and for the identification of
subjects having a predisposition to such disorders. Various forms of heart disease
include: cardiomyopathy, aortic valve prolapse; aortic valve stenosis; arrhythmia;
15 cardiogenic shock; congenital heart disease; heart attack; heart failure; heart tumor; heart
valve pulmonary stenosis; idiopathic cardiomyopathy; ischemic cardiomyopathy; mitral
regurgitation (acute); mitral regurgitation (chronic); mitral stenosis; mitral valve prolapse;
stable angina; hypotension; hypertension; acute heart failure; angina pectoris; and
tricuspid regurgitation.

20 Congestive heart failure may affect either the right side, left side, or both sides of
the heart. As pumping action is lost, blood may back up into other areas of the body,
including the liver, gastrointestinal tract, and extremities (right-sided heart failure), or the
lungs (left-sided heart failure).

25 Structural or functional causes of heart failure include high blood pressure
(hypertension), heart valve disease, congenital heart diseases, cardiomyopathy, heart
tumor, and other heart diseases. Precipitating factors include infections with high fever
or complicated infections, use of negative inotropic drugs (such as β -blocker and calcium
channel blocker), anemia, irregular heartbeats (arrhythmia), hyperthyroidism, and kidney
30 disease.

Furthermore, cardiomyopathy is a disease affecting the heart muscle
(myocardium); this disease usually results in the inadequate heart pumping. Causes,
incidence, and risk factors for cardiomyopathy include: viral infections; heart attacks;
alcoholism; long-term, severe high blood pressure (hypertension); or for other reasons
35 not yet known. Specific types of cardiomyopathy include: ischemic cardiomyopathy;
idiopathic cardiomyopathy; hypertrophic cardiomyopathy; alcoholic cardiomyopathy;
peripartum cardiomyopathy; dilated cardiomyopathy; and restrictive cardiomyopathy.

Cardiomyopathy is not common but can be severely disabling or fatal. Extreme
5 cardiomyopathy with heart failure may require a heart transplant.

Methods of diagnosing or detecting heart diseases may, for example, utilize
reagents such as the HGPRBMY2 nucleic acid sequences described in Section 5.1, and
HGPRBMY2 antibodies, as described, in Section 5.3. Specifically, such reagents may be
used, for example, for: (1) the detection of the presence of HGPRBMY2 gene mutations,
10 or the detection of either over- or under-expression of HGPRBMY2 mRNA relative to
the non-cardiovascular disorder state; (2) the detection of either an over- or an
under-abundance of HGPRBMY2 gene product relative to the non-cardiovascular
disorder state; and (3) the detection of perturbations or abnormalities in the signal
15 transduction pathway mediated by HGPRBMY2.

The methods described herein may be performed, for example, by utilizing
pre-packaged diagnostic kits comprising at least one specific HGPRBMY2 nucleic acid
sequence or HGPRBMY2 antibody reagent described herein, which may be conveniently
used, *e.g.*, in clinical settings, to diagnose patients exhibiting cardiovascular disorder
20 abnormalities.

For the detection of HGPRBMY2 mutations, any nucleated cell can be used as
a starting source for genomic nucleic acid. For the detection of HGPRBMY2 gene
expression or HGPRBMY2 gene products, any cell type or tissue in which the
HGPRBMY2 gene is expressed, such as, for example, heart cells, may be utilized.
25

Nucleic acid-based detection techniques are described, below, in Section 5.4.1.
Peptide detection techniques are described, below, in Section 5.4.2.

5.4.1. Detection of the HGPRBMY1 and HGPRBMY2 Gene and Transcripts

30 Mutations within the HGPRBMY1 or HGPRBMY2 gene can be detected by
utilizing a number of techniques. Nucleic acid from any nucleated cell can be used as the
starting point for such assay techniques, and may be isolated according to standard
nucleic acid preparation procedures which are well known to those of skill in the art.

35 DNA may be used in hybridization or amplification assays of biological samples
to detect abnormalities involving HGPRBMY1 or HGPRBMY2 gene structure, including
point mutations, insertions, deletions and chromosomal rearrangements. Such assays may

include, but are not limited to, Southern analyses, single stranded conformational
5 polymorphism analyses (SSCP), and PCR analyses.

Such diagnostic methods for the detection of HGPRBMY1 or HGPRBMY2
gene-specific mutations can involve for example, contacting and incubating nucleic acids
including recombinant DNA molecules, cloned genes or degenerate variants thereof,
10 obtained from a sample, *e.g.*, derived from a patient sample or other appropriate cellular
source, with one or more labeled nucleic acid reagents including recombinant DNA
molecules, cloned genes or degenerate variants thereof, as described in Section 5.1, under
conditions favorable for the specific annealing of these reagents to their complementary
sequences within the HGPRBMY1 or HGPRBMY2 gene. Preferably, the lengths of these
15 nucleic acid reagents are at least 15 to 30 nucleotides. After incubation, all non-annealed
nucleic acids are removed from the nucleic acid:HGPRBMY1 or HGPRBMY2 molecule
hybrid.

The presence of nucleic acids which have hybridized, if any such molecules exist,
is then detected. Using such a detection scheme, the nucleic acid from the cell type or
20 tissue of interest can be immobilized, for example, to a solid support such as a
membrane, or a plastic surface such as that on a microtiter plate or polystyrene beads. In
this case, after incubation, non-annealed, labeled nucleic acid reagents of the type
described in Section 5.1 are easily removed. Detection of the remaining, annealed,
25 labeled HGPRBMY1 or HGPRBMY2 nucleic acid reagents is accomplished using
standard techniques well-known to those in the art. The HGPRBMY1 or HGPRBMY2
gene sequences to which the nucleic acid reagents have annealed can be compared to the
annealing pattern expected from a normal HGPRBMY1 or HGPRBMY2 gene sequence
in order to determine whether an HGPRBMY1 or HGPRBMY2 gene mutation is present.

30 Alternative diagnostic methods for the detection of HGPRBMY1 or HGPRBMY2
gene specific nucleic acid molecules, in patient samples or other appropriate cell sources,
may involve their amplification, *e.g.*, by PCR (the experimental embodiment set forth in
Mullis, K. B., 1987, U.S. Pat. No. 4,683,202), followed by the detection of the amplified
35 molecules using techniques well known to those of skill in the art. The resulting
amplified sequences can be compared to those which would be expected if the nucleic
acid being amplified contained only normal copies of the HGPRBMY1 or HGPRBMY2

gene in order to determine whether an HGPRBMY1 or HGPRBMY2 gene mutation
5 exists.

Additionally, well-known genotyping techniques can be performed to identify
individuals carrying HGPRBMY1 or HGPRBMY2 gene mutations. Such techniques
include, for example, the use of restriction fragment length polymorphisms (RFLPs),
10 which involve sequence variations in one of the recognition sites for the specific
restriction enzyme used.

Additionally, improved methods for analyzing DNA polymorphisms which can
be utilized for the identification of HGPRBMY1 or HGPRBMY2 gene mutations have
been described which capitalize on the presence of variable numbers of short, tandemly
15 repeated DNA sequences between the restriction enzyme sites. For example, Weber (U.S.
Pat. No. 5,075,217, which is incorporated herein by reference in its entirety) describes a
DNA marker based on length polymorphisms in blocks of (dC-dA)_n-(dG-dT)_n short
tandem repeats. The average separation of (dC-dA)_n-(dG-dT)_n blocks is estimated to be
30,000-60,000 bp. Markers which are so closely spaced exhibit a high frequency
20 co-inheritance, and are extremely useful in the identification of genetic mutations, such
as, for example, mutations within the HGPRBMY1 or HGPRBMY2 gene, and the
diagnosis of diseases and disorders related to HGPRBMY1 or HGPRBMY2 mutations.

A DNA profiling assay for detecting short tri and tetra nucleotide repeat
25 sequences has been described (U.S. Pat. No. 5,364,759, which is incorporated herein by
reference in its entirety). This process includes extracting the DNA of interest, such as
the HGPRBMY1 or HGPRBMY2 gene, amplifying the extracted DNA, and labeling the
repeat sequences to form a genotypic map of the individual's DNA.

The level of HGPRBMY1 or HGPRBMY2 gene expression can also be assayed
30 by detecting and measuring HGPRBMY1 or HGPRBMY2 transcription. For example,
RNA from a cell type or tissue known, or suspected to express the HGPRBMY1 or
HGPRBMY2 gene, such as bone marrow or spleen cells, may be isolated and tested
utilizing hybridization or PCR techniques such as are described, above. The isolated cells
can be derived from cell culture or from a patient. The analysis of cells taken from culture
35 may be a necessary step in the assessment of cells to be used as part of a cell-based gene
therapy technique or, alternatively, to test the effect of compounds on the expression of
the HGPRBMY1 or HGPRBMY2 gene. Such analyses may reveal both quantitative and

qualitative aspects of the expression pattern of the HGPRBMY1 or HGPRBMY2 gene,
5 including activation or inactivation of HGPRBMY1 or HGPRBMY2 gene expression.

In one embodiment of such a detection scheme, cDNAs are synthesized from the
RNAs of interest (*e.g.*, by reverse transcription of the RNA molecule into cDNA). A
sequence within the cDNA is then used as the template for a nucleic acid amplification
reaction, such as a PCR amplification reaction, or the like. The nucleic acid reagents used
10 as synthesis initiation reagents (*e.g.*, primers) in the reverse transcription and nucleic acid
amplification steps of this method are chosen from among the HGPRBMY1 or
HGPRBMY2 nucleic acid reagents described in Section 5.1. The preferred lengths of
such nucleic acid reagents are at least 9-30 nucleotides. For detection of the amplified
15 product, the nucleic acid amplification may be performed using radioactively or
non-radioactively labeled nucleic acids. Alternatively, enough amplified product may be
made such that the product may be visualized by standard ethidium bromide staining or
by utilizing any other suitable nucleic acid staining method.

Additionally, it is possible to perform such HGPRBMY1 or HGPRBMY2 gene
20 expression assays "*in situ*", *i.e.*, directly upon tissue sections (fixed and/or frozen) of
patient tissue obtained from biopsies or resections, such that no nucleic acid purification
is necessary. Nucleic acid reagents such as those described in Section 5.1 may be used
as probes and/or primers for such *in situ* procedures (See, for example, Nuovo, G. J.,
25 1992, "PCR In Situ Hybridization: Protocols And Applications", Raven Press, NY).

Alternatively, if a sufficient quantity of the appropriate cells can be obtained,
standard Northern analysis can be performed to determine the level of mRNA expression
of the HGPRBMY1 or HGPRBMY2 gene.

30 5.4.2. Detection of the HGPRBMY1 and HGPRBMY2 Gene Products

Antibodies directed against wild type or mutant HGPRBMY1 or HGPRBMY2
gene products or conserved variants of the polypeptides or peptides, which are discussed,
above, in Section 5.3, may also be used as immune related disorder diagnostics and
prognostics, as described herein. Such diagnostic methods, may be used to detect
35 abnormalities in the level of HGPRBMY1 or HGPRBMY2 gene expression, or
abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of

the HGPRBMY1 or HGPRBMY2, and may be performed *in vivo* or *in vitro*, such as, for
5 example, on biopsy tissue.

For example, antibodies directed to epitopes of the HGPRBMY1 or HGPRBMY2
ECD can be used *in vivo* to detect the pattern and level of expression of the HGPRBMY1
or HGPRBMY2 in the body. Such antibodies can be labeled, *e.g.*, with a radio-opaque
or other appropriate compound and injected into a subject in order to visualize binding
10 to the HGPRBMY1 or HGPRBMY2 expressed in the body using methods such as
X-rays, CAT-scans, or MRI. Labeled antibody fragments, *e.g.*, the Fab or single chain
antibody comprising the smallest portion of the antigen binding region, are preferred for
maximum labeling of HGPRBMY1 or HGPRBMY2 expressed in the bone marrow or
15 spleen.

Additionally, any HGPRBMY1 or HGPRBMY2 fusion polypeptide or
HGPRBMY1 or HGPRBMY2 conjugated polypeptide whose presence can be detected,
can be administered. For example, HGPRBMY1 or HGPRBMY2 fusion or conjugated
polypeptides labeled with a radio-opaque or other appropriate compound can be
20 administered and visualized *in vivo* for labeled antibodies. Further such agonist or
antagonist fusion polypeptides as AP-GPCR or GPCR-Ap fusion polypeptides can be
utilized for *in vitro* diagnostic procedures. Alternatively, immunoassays or fusion
polypeptide detection assays, can be utilized on biopsy and autopsy samples *in vitro* to
25 permit assessment of the expression pattern of the HGPRBMY1 or HGPRBMY2. Such
assays are not confined to the use of antibodies that define the HGPRBMY1 or
HGPRBMY2 ECD, but can include the use of antibodies directed to epitopes of any of
the domains of the HGPRBMY1 or HGPRBMY2, *e.g.*, the ECD, the TM and/or CD. The
use of each or all of these labeled antibodies will yield useful information regarding
30 translation and intracellular transport of the HGPRBMY1 or HGPRBMY2 to the cell
surface, and can identify defects in processing.

The tissue or cell type to be analyzed will generally include those which are
known, or suspected, to express the HGPRBMY1 or HGPRBMY2 gene, such as, for
example, bone marrow or spleen cells. The polypeptide isolation methods employed
35 herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and
Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory
Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its

entirety. The isolated cells can be derived from cell culture or from a patient. The analysis
5 of cells taken from culture may be a necessary step in the assessment of cells that could
be used as part of a cell-based gene therapy technique or, alternatively, to test the effect
of compounds on the expression of the HGPRBMY1 or HGPRBMY2 gene. For example,
antibodies, or fragments of antibodies, such as those described, above, in Section 5.3,
10 useful in the present invention may be used to quantitatively or qualitatively detect the
presence of HGPRBMY1 or HGPRBMY2 gene products or conserved variants of the
polypeptides or peptides. This can be accomplished, for example, by
immunofluorescence techniques employing a fluorescently labeled antibody (see below,
this Section) coupled with light microscopic, flow cytometric, or fluorimetric detection.
15 Such techniques are especially preferred if such HGPRBMY1 or HGPRBMY2 gene
products are expressed on the cell surface.

The antibodies (or fragments thereof) or agonist or antagonist fusion or
conjugated polypeptides useful in the present invention may, additionally, be employed
histologically, as in immunofluorescence, immunoelectron microscopy or non-immuno
20 assays, for *in situ* detection of HGPRBMY1 or HGPRBMY2 gene products or conserved
variants of the polypeptides or peptides, or for agonist or antagonist binding (in the case
of labeled agonist or antagonist fusion polypeptide).

In situ detection may be accomplished by removing a histological specimen from
a patient, and applying thereto a labeled antibody or fusion polypeptide of the present
25 invention. The antibody (or fragment) or fusion polypeptide is preferably applied by
overlaying the labeled antibody (or fragment) onto a biological sample. Through the use
of such a procedure, it is possible to determine not only the presence of the HGPRBMY1
or HGPRBMY2 gene product, or conserved variants of the polypeptides or peptides, or
30 agonist or antagonist binding, but also its distribution in the examined tissue. Using the
present invention, those of ordinary skill will readily perceive that any of a wide variety
of histological methods (such as staining procedures) can be modified in order to achieve
such *in situ* detection.

Immunoassays and non-immunoassays for HGPRBMY1 or HGPRBMY2 gene
35 products or conserved variants of the polypeptides or peptides will typically comprise
incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells,
or lysates of cells which have been incubated in cell culture, in the presence of a

detectably labeled antibody capable of identifying HGPRBMY1 or HGPRBMY2 gene
5 products or conserved variants of the polypeptides or peptides, and detecting the bound
antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a
solid phase support or carrier such as nitrocellulose, or other solid support which is
capable of immobilizing cells, cell particles or soluble polypeptides. The support may
10 then be washed with suitable buffers followed by treatment with the detectably labeled
HGPRBMY1 or HGPRBMY2 antibody or agonist or antagonist fusion polypeptide. The
solid phase support may then be washed with the buffer a second time to remove
unbound antibody or fusion polypeptide. The amount of bound label on solid support may
15 then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an
antigen or an antibody. Well-known supports or carriers include glass, polystyrene,
polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses,
polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble
20 to some extent or insoluble for the purposes of the present invention. The support
material may have virtually any possible structural configuration so long as the coupled
molecule is capable of binding to an antigen or antibody. Thus, the support configuration
may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the
external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip,
25 etc. Preferred supports include polystyrene beads. Those skilled in the art will know many
other suitable carriers for binding antibody or antigen, or will be able to ascertain the
same by use of routine experimentation. The binding activity of a given lot of
HGPRBMY1 or HGPRBMY2 antibody or agonist or antagonist fusion polypeptide may
30 be determined according to well known methods.

Those skilled in the art will be able to determine optimal assay conditions for
each determination by employing routine experimentation.

With respect to antibodies, one of the ways in which the HGPRBMY1 or
HGPRBMY2 antibody can be detectably labeled is by linking the same to an enzyme and
35 used in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent
Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly
Publication, Walkersville, Md.); Voller, A. et al., 1978, J. Clin. Pathol. 31:507-520;

Butler, J. E., 1981, *Meth. Enzymol.* 73:482-523; Maggio, E. (ed.), 1980, *Enzyme*
5 *Immunoassay*, CRC Press, Boca Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, *Enzyme*
Immunoassay, Kaku Shoin, Tokyo).

The enzyme which is bound to the antibody will react with an appropriate
substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical
moiety which can be detected, for example, by spectrophotometric, fluorimetric or by
10 visual means. Enzymes which can be used to detectably label the antibody include, but
are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid
isomerase, yeast alcohol dehydrogenase, alphas-glycerophosphate, dehydrogenase, triose
phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose
15 oxidase, β -galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate
dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be
accomplished by calorimetric methods which employ a chromogenic substrate for the
enzyme. Detection may also be accomplished by visual comparison of the extent of
enzymatic reaction of a substrate in comparison with similarly prepared standards.

20 Detection may also be accomplished using any of a variety of other
immunoassays. For example, by radioactively labeling the antibodies or antibody
fragments, it is possible to detect HGPRBMY1 or HGPRBMY2 through the use of a
radioimmunoassay (RIA) (see, for example, Weintraub, B., *Principles of*
Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The
25 Endocrine Society, March, 1986, which is incorporated by reference herein). The
radioactive isotope can be detected by such means as the use of a gamma counter or a
scintillation counter or by autoradiography.

It is also possible to label the antibody with a fluorescent compound. When the
30 fluorescently labeled antibody is exposed to light of the proper wave length, its presence
can then be detected due to fluorescence. Among the most commonly used fluorescent
labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin,
phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals
35 such as ^{152}Eu , or others of the lanthanide series. These metals can be attached to the
antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA)
or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent
5 compound. The presence of the chemiluminescent-tagged antibody is then determined
by detecting the presence of luminescence that arises during the course of a chemical
reaction. Examples of particularly useful chemiluminescent labeling compounds are
luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate
10 ester.

Likewise, a bioluminescent compound may be used to label the antibody of the
present invention. Bioluminescence is a type of chemiluminescence found in biological
systems in, which a catalytic polypeptide increases the efficiency of the
chemiluminescent reaction. The presence of a bioluminescent polypeptide is determined
15 by detecting the presence of luminescence. Important bioluminescent compounds for
purposes of labeling are luciferin, luciferase and aequorin.

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5 5.5. Screening Assays for Compounds that Modulate HGPRBMY1 and HGPRBMY2

The following assays are designed to identify compounds that interact with (*e.g.*, bind to) HGPRBMY1 or HGPRBMY2 (including, but not limited to the ECD or CD of HGPRBMY1 or HGPRBMY2), compounds that interact with (*e.g.*, bind to) intracellular
10 polypeptides that interact with HGPRBMY1 or HGPRBMY2 (including, but not limited to, the TM and CD of HGPRBMY1 or HGPRBMY2), compounds that interfere with the interaction of HGPRBMY1 or HGPRBMY2 with transmembrane or intracellular polypeptides involved in HGPRBMY1 or HGPRBMY2-mediated signal transduction,
15 and to compounds which modulate the activity of HGPRBMY1 or HGPRBMY2 gene (*i.e.*, modulate the level of HGPRBMY1 or HGPRBMY2 gene expression) or modulate the level of HGPRBMY1 or HGPRBMY2. Assays may additionally be utilized which identify compounds which bind to HGPRBMY1 or HGPRBMY2 gene regulatory sequences (*e.g.*, promoter sequences) and which may modulate HGPRBMY1 or
20 HGPRBMY2 gene expression. See *e.g.*, Platt, K. A., 1994, J. Biol. Chem.. 269:28558-28562, which is incorporated herein by reference in its entirety.

The compounds which may be screened in accordance with the invention include, but are not limited to peptides, antibodies and fragments thereof, and other organic
25 compounds (*e.g.*, peptidomimetics) that bind to the ECD of the HGPRBMY1 or HGPRBMY2 and either mimic the activity triggered by the natural ligand (*i.e.*, agonists) or inhibit the activity triggered by the natural ligand (*i.e.*, antagonists); as well as peptides, antibodies or fragments thereof, and other organic compounds that mimic the ECD of the HGPRBMY1 or HGPRBMY2 (or a portion thereof) and bind to and
30 "neutralize" natural ligand.

Such compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to members of random peptide libraries; (see, *e.g.*, Lam, K. S. et al., 1991, Nature 354:82-84; Houghten, R. et al., 1991, Nature 354:84-86), and combinatorial chemistry-derived molecular library made of D-
35 and/or L- configuration amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate, directed phosphopeptide libraries; see, *e.g.*, Songyang, Z. et al., 1993, Cell 72:767-778), antibodies (including, but not limited to,

polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies,
5 and FAb, F(ab')₂ and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

Other compounds which can be screened in accordance with the invention include but are not limited to small organic molecules which may gain entry into an appropriate cell (*e.g.*, in the bone marrow or spleen) and affect the expression of the
10 HGPRBMY1 gene or some other gene involved in the HGPRBMY1 signal transduction pathway (*e.g.*, by interacting with the regulatory region or transcription factors involved in gene expression); or such compounds that affect the activity of the HGPRBMY1 (*e.g.*, by inhibiting or enhancing the enzymatic activity of the CD) or the activity of some other
15 intracellular factor involved in the HGPRBMY1 signal transduction pathway, such as, for example, gp130.

Other compounds which can be screened in accordance with the invention include but are not limited to small organic molecules which may gain entry into an appropriate cell (*e.g.*, in the heart) and affect the expression of the HGPRBMY2 gene or
20 some other gene involved in the HGPRBMY2 signal transduction pathway (*e.g.*, by interacting with the regulatory region or transcription factors involved in gene expression); or such compounds that affect the activity of the HGPRBMY2 (*e.g.*, by inhibiting or enhancing the enzymatic activity of the CD) or the activity of some other
25 intracellular factor involved in the HGPRBMY2 signal transduction pathway, such as, for example, gp130.

Computer modelling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can modulate HGPRBMY1 or HGPRBMY2 expression or activity. Having identified such a compound
30 or composition, the active sites or regions are identified. Such active sites might typically be ligand binding sites, such as the interaction domains of agonist or antagonist with HGPRBMY1 or HGPRBMY2 itself. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from
35 the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the complexed ligand is found. Next, the three dimensional geometric structure of the

active site is determined. This can be done by known methods, including X-ray
5 crystallography, which can determine a complete molecular structure. On the other hand,
solid or liquid phase NMR can be used to determine certain intra-molecular distances.
Any other experimental method of structure determination can be used to obtain partial
or complete geometric structures. The geometric structures may be measured with a
10 complexed ligand, natural or artificial, which may increase the accuracy of the active site
structure determined.

If an incomplete or insufficiently accurate structure is determined, the methods
of computer based numerical modelling can be used to complete the structure or improve
its accuracy. Any recognized modelling method may be used, including parameterized
15 models specific to particular biopolymers such as polypeptides or nucleic acids,
molecular dynamics models based on computing molecular motions, statistical mechanics
models based on thermal ensembles, or combined models. For most types of models,
standard molecular force fields, representing the forces between constituent atoms and
groups, are necessary, and can be selected from force fields known in physical chemistry.
20 The incomplete or less accurate experimental structures can serve as constraints on the
complete and more accurate structures computed by these modeling methods.

Finally, having determined the structure of the active site, either experimentally,
by modeling, or by a combination, candidate modulating compounds can be identified
25 by searching databases containing compounds along with information on their molecular
structure. Such a search seeks compounds having structures that match the determined
active site structure and that interact with the groups defining the active site. Such a search
can be manual, but is preferably computer assisted. These compounds found from this
search are potential HGPRBMY1 or HGPRBMY2 modulating compounds.

30 Alternatively, these methods can be used to identify improved modulating
compounds from an already known modulating compound or ligand. The composition
of the known compound can be modified and the structural effects of modification can
be determined using the experimental and computer modelling methods described above
applied to the new composition. The altered structure is then compared to the active site
35 structure of the compound to determine if an improved fit or interaction results. In this
manner systematic variations in composition, such as by varying side groups, can be

quickly evaluated to obtain modified modulating compounds or ligands of improved
5 specificity or activity.

Further experimental and computer modeling methods useful to identify
modulating compounds based upon identification of the active sites of agonist or
antagonist, HGPRBMY1 or HGPRBMY2, and related transduction and transcription
factors will be apparent to those of skill in the art.

10 Examples of molecular modelling systems are the CHARMM and QUANTA
programs (Polygen Corporation, Waltham, Mass.). CHARMM performs the energy
minimization and molecular dynamics functions. QUANTA performs the construction,
graphic modelling and analysis of molecular structure. QUANTA allows interactive
15 construction, modification, visualization, and analysis of the behavior of molecules with
each other.

A number of articles review computer modeling of drugs interactive with
specific-polypeptides, such as Rotivinen, et al., 1988, *Acta Pharmaceutica Fennica*
97:159-166; Ripka, *New Scientist* 54-57 (Jun. 16, 1988); McKinaly and Rossmann, 1989,
20 *Annu. Rev. Pharmacol. Toxicol.* 29:111-122; Perry and Davies, *OSAR: Quantitative*
Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989);
Lewis and Dean, 1989 *Proc. R. Soc. Lond.* 236:125-140 and 141-162; and, with respect
to a model receptor for nucleic acid components, Askew, et al., 1989, *J. Am. Chem. Soc.*
25 111:1082-1090. Other computer programs that screen and graphically depict chemicals
are available from companies such as BioDesign, Inc. (Pasadena, Calif.), Allelix, Inc.
(Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although
these are primarily designed for application to drugs specific to particular polypeptides,
they can be adapted to design of drugs specific to regions of DNA or RNA, once that
30 region is identified.

Although described above with reference to design and generation of compounds
which could alter binding, one could also screen libraries of known compounds,
including natural products or synthetic chemicals, and biologically active materials,
including polypeptides, for compounds which are inhibitors or activators.

35 Compounds identified via assays such as those described herein may be useful,
for example, in elaborating the biological function of the HGPRBMY1 gene product, and
for ameliorating immune disorders. Assays for testing the effectiveness of compounds,

identified by, for example, techniques such as those described in Section 5.5.1 through
5 5.5.3, are discussed, below, in Section 5.5.4.

Compounds identified via assays such as those described herein may be useful,
for example, in elaborating the biological function of the HGPRBMY2 gene product, and
for ameliorating cardiovascular disorders. Assays for testing the effectiveness of
compounds, identified by, for example, techniques such as those described in Section
10 5.5.1 through 5.5.3, are discussed, below, in Section 5.5.4.

The human HGPRBMY1 or HGPRBMY2 polypeptides and/or peptides of the
present invention, or immunogenic fragments or oligopeptides thereof, can be used for
screening therapeutic drugs or compounds in a variety of drug screening techniques. The
15 fragment employed in such a screening assay may be free in solution, affixed to a solid
support, borne on a cell surface, or located intracellularly. The reduction or abolition of
activity of the formation of binding complexes between the ion channel protein and the
agent being tested can be measured. Thus, the present invention provides a method for
screening or assessing a plurality of compounds for their specific binding affinity with
20 a HGPRBMY1 or HGPRBMY2 polypeptide, or a bindable peptide fragment, of this
invention, comprising providing a plurality of compounds, combining the HGPRBMY1
or HGPRBMY2 polypeptide, or a bindable peptide fragment, with each of a plurality of
compounds for a time sufficient to allow binding under suitable conditions and detecting
25 binding of the HGPRBMY1 or HGPRBMY2 polypeptide or peptide to each of the
plurality of test compounds, thereby identifying the compounds that specifically bind to
the HGPRBMY1 or HGPRBMY2 polypeptide or peptide.

Methods of identifying compounds that modulate the activity of the novel human
HGPRBMY1 or HGPRBMY2 polypeptides and/or peptides are provided by the present
30 invention and comprise combining a potential or candidate compound or drug modulator
of G-protein coupled receptor biological activity with an HGPRBMY1 or HGPRBMY2
polypeptide or peptide, for example, the HGPRBMY1 or HGPRBMY2 amino acid
sequence as set forth in SEQ ID NO:2 or SEQ ID NO:14, and measuring an effect of the
candidate compound or drug modulator on the biological activity of the HGPRBMY1 or
35 HGPRBMY2 polypeptide or peptide. Such measurable effects include, for example,
physical binding interaction; the ability to cleave a suitable G-protein coupled receptor
substrate; effects on native and cloned HGPRBMY1 or HGPRBMY2-expressing cell

line; and effects of modulators or other G-protein coupled receptor-mediated
5 physiological measures.

Another method of identifying compounds that modulate the biological activity
of the novel HGPRBMY1 or HGPRBMY2 polypeptides of the present invention
comprises combining a potential or candidate compound or drug modulator of a G-
protein coupled receptor biological activity with a host cell that expresses the
10 HGPRBMY1 or HGPRBMY2 polypeptide and measuring an effect of the candidate
compound or drug modulator on the biological activity of the HGPRBMY1 or
HGPRBMY2 polypeptide. The host cell can also be capable of being induced to express
the HGPRBMY1 or HGPRBMY2 polypeptide, e.g., via inducible expression.
15 Physiological effects of a given modulator candidate on the HGPRBMY1 or
HGPRBMY2 polypeptide can also be measured. Thus, cellular assays for particular G-
protein coupled receptor modulators may be either direct measurement or quantification
of the physical biological activity of the HGPRBMY1 or HGPRBMY2 polypeptide, or
they may be measurement or quantification of a physiological effect. Such methods
20 preferably employ a HGPRBMY1 or HGPRBMY2 polypeptide as described herein, or
an overexpressed recombinant HGPRBMY1 or HGPRBMY2 polypeptide in suitable host
cells containing an expression vector as described herein, wherein the HGPRBMY1 or
HGPRBMY2 polypeptide is expressed, overexpressed, or undergoes upregulated
expression.
25

Another aspect of the present invention embraces a method of screening for a
compound that is capable of modulating the biological activity of a HGPRBMY1 or
HGPRBMY2 polypeptide, comprising providing a host cell containing an expression
vector harboring a nucleic acid sequence encoding a HGPRBMY1 or HGPRBMY2
30 polypeptide, or a functional peptide or portion thereof (e.g., SEQ ID NOS:2); determining
the biological activity of the expressed HGPRBMY1 or HGPRBMY2 polypeptide in the
absence of a modulator compound; contacting the cell with the modulator compound and
determining the biological activity of the expressed HGPRBMY1 or HGPRBMY2
polypeptide in the presence of the modulator compound. In such a method, a difference
35 between the activity of the HGPRBMY1 or HGPRBMY2 polypeptide in the presence of
the modulator compound and in the absence of the modulator compound indicates a
modulating effect of the compound.

Essentially any chemical compound can be employed as a potential modulator or
5 ligand in the assays according to the present invention. Compounds tested as G-protein
coupled receptor modulators can be any small chemical compound, or biological entity
(e.g., protein, sugar, nucleic acid, lipid). Test compounds will typically be small chemical
molecules and peptides. Generally, the compounds used as potential modulators can be
10 dissolved in aqueous or organic (e.g., DMSO-based) solutions. The assays are designed
to screen large chemical libraries by automating the assay steps and providing compounds
from any convenient source. Assays are typically run in parallel, for example, in
microtiter formats on microtiter plates in robotic assays. There are many suppliers of
chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-
15 Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland),
for example. Also, compounds may be synthesized by methods known in the art.

High throughput screening methodologies are particularly envisioned for the
detection of modulators of the novel HGPRBMY1 or HGPRBMY2 polynucleotides and
polypeptides described herein. Such high throughput screening methods typically involve
20 providing a combinatorial chemical or peptide library containing a large number of
potential therapeutic compounds (e.g., ligand or modulator compounds). Such
combinatorial chemical libraries or ligand libraries are then screened in one or more
assays to identify those library members (e.g., particular chemical species or subclasses)
25 that display a desired characteristic activity. The compounds so identified can serve as
conventional lead compounds, or can themselves be used as potential or actual
therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds
generated either by chemical synthesis or biological synthesis, by combining a number
30 of chemical building blocks (i.e., reagents such as amino acids). As an example, a linear
combinatorial library, e.g., a polypeptide or peptide library, is formed by combining a set
of chemical building blocks in every possible way for a given compound length (i.e., the
number of amino acids in a polypeptide or peptide compound). Millions of chemical
compounds can be synthesized through such combinatorial mixing of chemical building
35 blocks.

The preparation and screening of combinatorial chemical libraries is well known
to those having skill in the pertinent art. Combinatorial libraries include, without

limitation, peptide libraries (e.g. U.S. Patent No. 5,010,175; Furka, 1991, *Int. J. Pept. Prot. Res.*, 37:487-493; and Houghton et al., 1991, *Nature*, 354:84-88). Other chemistries for generating chemical diversity libraries can also be used. Nonlimiting examples of chemical diversity library chemistries include, peptides (PCT Publication No. WO 91/019735), encoded peptides (PCT Publication No. WO 93/20242), random bio-oligomers (PCT Publication No. WO 92/00091), benzodiazepines (U.S. Patent No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:6909-6913), vinylogous polypeptides (Hagihara et al., 1992, *J. Amer. Chem. Soc.*, 114:6568), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., 1992, *J. Amer. Chem. Soc.*, 114:9217-9218), analogous organic synthesis of small compound libraries (Chen et al., 1994, *J. Amer. Chem. Soc.*, 116:2661), oligocarbamates (Cho et al., 1993, *Science*, 261:1303), and/or peptidyl phosphonates (Campbell et al., 1994, *J. Org. Chem.*, 59:658), nucleic acid libraries (see Ausubel, Berger and Sambrook, all supra), peptide nucleic acid libraries (U.S. Patent No. 5,539,083), antibody libraries (e.g., Vaughn et al., 1996, *Nature Biotechnology*, 14(3):309-314) and PCT/US96/10287), carbohydrate libraries (e.g., Liang et al., 1996, *Science*, 274:1520-1522) and U.S. Patent No. 5,593,853), small organic molecule libraries (e.g., benzodiazepines, Baum C&EN, Jan. 18, 1993, page 33; and U.S. Patent No. 5,288,514; isoprenoids, U.S. Patent No. 5,569,588; thiazolidinones and metathiazanones, U.S. Patent No. 5,549,974; pyrrolidines, U.S. Patent Nos. 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent No. 5,506,337; and the like).

Devices for the preparation of combinatorial libraries are commercially available (e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY; Symphony, Rainin, Woburn, MA; 433A Applied Biosystems, Foster City, CA; 9050 Plus, Millipore, Bedford, MA). In addition, a large number of combinatorial libraries are commercially available (e.g., ComGenex, Princeton, NJ; Asinex, Moscow, Russia; Tripos, Inc., St. Louis, MO; ChemStar, Ltd., Moscow, Russia; 3D Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD, and the like).

In one embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the cell or tissue expressing an ion channel is attached to a solid phase substrate. In such high throughput assays, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well

of a microtiter plate can be used to perform a separate assay against a selected potential
5 modulator, or, if concentration or incubation time effects are to be observed, every 5-10
wells can test a single modulator. Thus, a single standard microtiter plate can assay about
96 modulators. If 1536 well plates are used, then a single plate can easily assay from
about 100 to about 1500 different compounds. It is possible to assay several different
10 plates per day; thus, for example, assay screens for up to about 6,000-20,000 different
compounds are possible using the described integrated systems.

In another of its aspects, the present invention encompasses screening and small
molecule (e.g., drug) detection assays which involve the detection or identification of
small molecules that can bind to a given protein, i.e., a HGPRBMY1 or HGPRBMY2
15 polypeptide or peptide. Particularly preferred are assays suitable for high throughput
screening methodologies.

In such binding-based detection, identification, or screening assays, a functional
assay is not typically required. All that is needed is a target protein, preferably
substantially purified, and a library or panel of compounds (e.g., ligands, drugs, small
20 molecules) or biological entities to be screened or assayed for binding to the protein
target. Preferably, most small molecules that bind to the target protein will modulate
activity in some manner, due to preferential, higher affinity binding to functional areas
or sites on the protein.

An example of such an assay is the fluorescence based thermal shift assay (3-
25 Dimensional Pharmaceuticals, Inc., 3DP, Exton, PA) as described in U.S. Patent Nos.
6,020,141 and 6,036,920 to Pantoliano et al.; see also, J. Zimmerman, 2000, *Gen. Eng.
News*, 20(8)). The assay allows the detection of small molecules (e.g., drugs, ligands) that
bind to expressed, and preferably purified, ion channel polypeptide based on affinity of
30 binding determinations by analyzing thermal unfolding curves of protein-drug or ligand
complexes. The drugs or binding molecules determined by this technique can be further
assayed, if desired, by methods, such as those described herein, to determine if the
molecules affect or modulate function or activity of the target protein.

To purify a HGPRBMY1 or HGPRBMY2 polypeptide or peptide to measure a
35 biological binding or ligand binding activity, the source may be a whole cell lysate that
can be prepared by successive freeze-thaw cycles (e.g., one to three) in the presence of
standard protease inhibitors. The HGPRBMY1 or HGPRBMY2 polypeptide may be

partially or completely purified by standard protein purification methods, e.g., affinity
5 chromatography using specific antibody described *infra*, or by ligands specific for an
epitope tag engineered into the recombinant HGPRBMY1 or HGPRBMY2 polypeptide
molecule, also as described herein. Binding activity can then be measured as described.

Compounds which are identified according to the methods provided herein, and
which modulate or regulate the biological activity or physiology of the HGPRBMY1 or
10 HGPRBMY2 polypeptides according to the present invention are a preferred embodiment
of this invention. It is contemplated that such modulatory compounds may be employed
in treatment and therapeutic methods for treating a condition that is mediated by the
novel HGPRBMY1 or HGPRBMY2 polypeptides by administering to an individual in
15 need of such treatment a therapeutically effective amount of the compound identified by
the methods described herein.

In addition, the present invention provides methods for treating an individual in
need of such treatment for a disease, disorder, or condition that is mediated by the
HGPRBMY1 or HGPRBMY2 polypeptides of the invention, comprising administering
20 to the individual a therapeutically effective amount of the HGPRBMY1 or HGPRBMY2-
modulating compound identified by a method provided herein.

25 5.5.1. *In Vitro* Screening Assays for Compounds that Bind to HGPRBMY1 or HGPRBMY2

In vitro systems may be designed to identify compounds capable of interacting
with (e.g., binding to) HGPRBMY1 or HGPRBMY2 (including, but not limited to, the
ECD or CD of HGPRBMY1 or HGPRBMY2). Compounds identified may be useful, for
30 example, in modulating the activity of wild type and/or mutant HGPRBMY1 or
HGPRBMY2 gene products; may be useful in elaborating the biological function of the
HGPRBMY1 or HGPRBMY2; may be utilized in screens for identifying compounds that
disrupt normal HGPRBMY1 or HGPRBMY2 interactions; or may in themselves disrupt
such interactions.

35 The principle of the assays used to identify compounds that bind to the
HGPRBMY1 or HGPRBMY2 involves preparing a reaction mixture of the HGPRBMY1
or HGPRBMY2 and the test compound under conditions and for a time sufficient to

allow the two components to interact and bind, thus forming a complex which can be
5 removed and/or detected in the reaction mixture. The HGPRBMY1 or HGPRBMY2
species used can vary depending upon the goal of the screening assay. For example,
where agonists of the natural ligand are sought, the full length HGPRBMY1 or
HGPRBMY2, or a soluble truncated HGPRBMY1 or HGPRBMY2, *e.g.*, in which the
10 TM and/or CD is deleted from the molecule, a peptide corresponding to the ECD or a
fusion polypeptide containing the HGPRBMY1 or HGPRBMY2 ECD fused to a
polypeptide or peptide that affords advantages in the assay system (*e.g.*, labeling,
isolation of the resulting complex, etc.) can be utilized. Where compounds that interact
with the cytoplasmic domain are sought to be identified, peptides corresponding to the
15 HGPRBMY1 or HGPRBMY2 CD and fusion polypeptides containing the HGPRBMY1
or HGPRBMY2 CD can be used.

The screening assays can be conducted in a variety of ways. For example, one
method to conduct such an assay would involve anchoring the HGPRBMY1 or
HGPRBMY2 polypeptide, peptide or fusion polypeptide or the test substance onto a solid
20 phase and detecting HGPRBMY1 or HGPRBMY2/test compound complexes anchored
on the solid phase at the end of the reaction. In one embodiment of such a method, the
HGPRBMY1 or HGPRBMY2 reactant may be anchored onto a solid surface, and the test
compound, which is not anchored, may be labeled, either directly or indirectly.

25 In practice, microtiter plates may conveniently be utilized as the solid phase. The
anchored component may be immobilized by non-covalent or covalent attachments.
Non-covalent attachment may be accomplished by simply coating the solid surface with
a solution of the polypeptide and drying. Alternatively, an immobilized antibody,
preferably a monoclonal antibody, specific for the polypeptide to be immobilized may be
30 used to anchor the polypeptide to the solid surface. The surfaces may be prepared in
advance and stored.

In order to conduct the assay, the nonimmobilized component is added to the
coated surface containing the anchored component. After the reaction is complete,
unreacted components are removed (*e.g.*, by washing) under conditions such that any
35 complexes formed will remain immobilized on the solid surface. The detection of
complexes anchored on the solid surface can be accomplished in a number of ways.
Where the previously nonimmobilized component is pre-labeled, the detection of label

immobilized on the surface indicates that complexes were formed. Where the previously
5 nonimmobilized component is not pre-labeled, an indirect label can be used to detect
complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the
previously nonimmobilized component (the antibody, in turn, may be directly labeled or
indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products
10 separated from unreacted components, and complexes detected; *e.g.*, using an
immobilized antibody specific for HGPRBMY1 or HGPRBMY2 polypeptide, peptide
or fusion polypeptide or the test compound to anchor any complexes formed in solution,
and a labeled antibody specific for the other component of the possible complex to detect
15 anchored complexes.

Alternatively, cell-based assays can be used to identify compounds that interact
with HGPRBMY1 or HGPRBMY2. To this end, cell lines that express HGPRBMY1 or
HGPRBMY2, or cell lines (*e.g.*, COS cells, CHO cells, fibroblasts, etc.) that have been
genetically engineered to express HGPRBMY1 or HGPRBMY2 (*e.g.*, by transfection or
20 transduction of HGPRBMY1 or HGPRBMY2 DNA) can be used. Interaction of the test
compound with, for example, the ECD of HGPRBMY1 or HGPRBMY2 expressed by
the host cell can be determined by comparison or competition with native agonist or
antagonist.

25 5.5.2. Assays for Polypeptides that Interact with the HGPRBMY1 or HGPRBMY2

Any method suitable for detecting polypeptide-polypeptide interactions may be
employed for identifying transmembrane polypeptides or intracellular polypeptides that
interact with HGPRBMY1 or HGPRBMY2. Among the traditional methods which may
30 be employed are co-immunoprecipitation, crosslinking and co-purification through
gradients or chromatographic columns of cell lysates or polypeptides obtained from cell
lysates and the HGPRBMY1 or HGPRBMY2 to identify polypeptides in the lysate that
interact with the HGPRBMY1 or HGPRBMY2. For these assays, the HGPRBMY1 or
HGPRBMY2 component used can be a full length HGPRBMY1 or HGPRBMY2, a
35 soluble derivative lacking the membrane-anchoring region (*e.g.*, a truncated HGPRBMY1
or HGPRBMY2 in which the TM is deleted resulting in a truncated molecule containing
the ECD fused to the CD), a peptide corresponding to the CD or a fusion polypeptide

containing the CD of HGPRBMY1 or HGPRBMY2. Once isolated, such an intracellular
5 polypeptide can be identified and can, in turn, be used, in conjunction with standard
techniques, to identify polypeptides with which it interacts. For example, at least a
portion of the amino acid sequence of an intracellular polypeptide which interacts with
the HGPRBMY1 or HGPRBMY2 can be ascertained using techniques well known to
10 those of skill in the art, such as via the Edman degradation technique (See, *e.g.*,
Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman & Co.,
N.Y., pp.34-49). The amino acid sequence obtained may be used as a guide for the
generation of oligonucleotide mixtures that can be used to screen for gene sequences
encoding such intracellular polypeptides. Screening may be accomplished, for example,
15 by standard hybridization or PCR techniques. Techniques for the generation of
oligonucleotide mixtures and the screening are well-known (See, *e.g.*, Ausubel, *supra.*,
and PCR Protocols: A Guide to Methods and Applications, 1990, Innis, M. et al., eds.
Academic Press, Inc., New York).

Additionally, methods may be employed which result in the simultaneous
20 identification of genes which encode the transmembrane or intracellular polypeptides
interacting with HGPRBMY1 or HGPRBMY2. These methods include, for example,
probing expression, libraries, in a manner similar to the well known technique of
antibody probing of λ gt11 libraries, using labeled HGPRBMY1 or HGPRBMY2
25 polypeptide, or an HGPRBMY1 or HGPRBMY2 polypeptide, peptide or fusion
polypeptide, *e.g.*, an HGPRBMY1 or HGPRBMY2 polypeptide or HGPRBMY1 or
HGPRBMY2 domain fused to a marker (*e.g.*, an enzyme, fluor, luminescent polypeptide,
or dye), or an Ig-Fc domain.

One method which detects polypeptide interactions *in vivo*, the two-hybrid
30 system, is described in detail for illustration only and not by way of limitation. One
version of this system has been described (Chien et al., 1991, Proc. Natl. Acad. Sci. USA,
88:9578-9582) and is commercially available from Clontech (Palo Alto, Calif.).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid
polypeptides: one plasmid consists of nucleic acids encoding the DNA-binding domain
35 of a transcription activator polypeptide fused to an HGPRBMY1 or HGPRBMY2 nucleic
acid sequence encoding HGPRBMY1 or HGPRBMY2, an HGPRBMY1 or HGPRBMY2
polypeptide, peptide or fusion polypeptide, and the other plasmid consists of nucleic

acids encoding the transcription activator polypeptide's activation domain fused to a
5 cDNA encoding an unknown polypeptide which has been recombined into this plasmid
as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA
library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains
a reporter gene (*e.g.*, HBS or lacZ) whose regulatory region contains the transcription
10 activator's binding site. Either hybrid polypeptide alone cannot activate transcription of
the reporter gene: the DNA-binding domain hybrid cannot because it does not provide
activation function and the activation domain hybrid cannot because it cannot localize to
the activator's binding sites. Interaction of the two hybrid polypeptides reconstitutes the
functional activator polypeptide and results in expression of the reporter gene, which is
15 detected by an assay for the reporter gene product.

The two-hybrid system or related methodology may be used to screen activation
domain libraries for polypeptides that interact with the "bait" gene product. By way of
example, and not by way of limitation, HGPRBMY1 or HGPRBMY2 may be used as the
bait gene product. Total genomic or cDNA sequences are fused to the DNA encoding an
20 activation domain. This library and a plasmid encoding a hybrid of a bait HGPRBMY1
or HGPRBMY2 gene product fused to the DNA-binding domain are cotransformed into
a yeast reporter strain, and the resulting transformants are screened for those that express
the reporter gene. For example, and not by way of limitation, a bait HGPRBMY1 or
25 HGPRBMY2 gene sequence, such as the open reading frame of HGPRBMY1 or
HGPRBMY2 (or a domain of HGPRBMY1 or HGPRBMY2), as depicted in Figure 1 can
be cloned into a vector such that it is translationally fused to the DNA encoding the
DNA-binding domain of the GAL4 polypeptide. These colonies are purified and the
library plasmids responsible for reporter gene expression are isolated. DNA sequencing
30 is then used to identify the polypeptides encoded by the library plasmids.

A cDNA library of the cell line from which polypeptides that interact with bait
HGPRBMY1 or HGPRBMY2 gene product are to be detected can be made using
methods routinely practiced in the art. According to the particular system described
herein, for example, the cDNA fragments can be inserted into a vector such that they are
35 translationally fused to the transcriptional activation domain of GAL4. This library can
be co-transformed along with the bait HGPRBMY1 or HGPRBMY2 gene-GAL4 fusion
plasmid into a yeast strain which contains a lacZ gene driven by a promoter which

contains GAL4 activation sequence. A cDNA encoded polypeptide, fused to GAL4
5 transcriptional activation domain, that interacts with bait HGPRBMY1 or HGPRBMY2
gene product will reconstitute an active GAL4 polypeptide and thereby drive expression
of the HIS3 gene. Colonies which express HIS3 can be detected by their growth on petri
dishes containing semi-solid agar based media lacking histidine. The cDNA can then be
10 purified from these strains, and used to produce and isolate the bait HGPRBMY1 or
HGPRBMY2 gene-interacting polypeptide using techniques routinely practiced in the art.

Additional assays for identifying polypeptides that bind to and potentially
modulate the HGPRBMY1 or HGPRBMY2 polypeptides are described elsewhere herein.
More specifically, peptides have been identified that have been shown to bind to and
15 potentially modulate the HGPRBMY2 polypeptide.

5.5.3. Assays for other Compounds

The macromolecules that interact with the HGPRBMY1 are referred to, for
purposes of this discussion, as "binding partners". These binding partners are likely to be
20 involved in the HGPRBMY1 signal transduction pathway, and therefore, in the role of
HGPRBMY1 in immune related regulation. Therefore, it is desirable to identify
compounds that interfere with or disrupt the interaction of such binding partners with
agonist or antagonist which may be useful in regulating the activity of the HGPRBMY1
and control immune disorders associated with HGPRBMY1 activity.
25

The macromolecules that interact with the HGPRBMY2 are referred to, for
purposes of this discussion, as "binding partners". These binding partners are likely to be
involved in the HGPRBMY2 signal transduction pathway, and therefore, in the role of
HGPRBMY2 in cardiovascular regulation. Therefore, it is desirable to identify
30 compounds that interfere with or disrupt the interaction of such binding partners with
agonist or antagonist which may be useful in regulating the activity of the HGPRBMY2
and control cardiovascular or neural disorders associated with HGPRBMY2 activity.

The basic principle of the assay systems used to identify compounds that interfere
with the interaction between the HGPRBMY1 or HGPRBMY2 and its binding partner
35 or partners involves preparing a reaction mixture containing HGPRBMY1 or
HGPRBMY2 polypeptide, peptide or fusion polypeptide as described in Sections 5.5.1
and 5.5.2 above, and the binding partner under conditions and for a time sufficient to

allow the two to interact and bind, thus forming a complex. In order to test a compound
5 for inhibitory activity, the reaction mixture is prepared in the presence and absence of the
test compound. The test compound may be initially included in the reaction mixture, or
may be added at a time subsequent to the addition of the HGPRBMY1 or HGPRBMY2
moiety and its binding partner. Control reaction mixtures are incubated without the test
10 compound or with a placebo. The formation of any complexes between the HGPRBMY1
or HGPRBMY2 moiety and the binding partner is then detected. The formation of a
complex in the control reaction, but not in the reaction mixture containing the test
compound, indicates that the compound interferes with the interaction of the.
HGPRBMY1 or HGPRBMY2 and the interactive binding partner. Additionally, complex
15 formation within reaction mixtures containing the test compound and normal
HGPRBMY1 or HGPRBMY2 polypeptide may also be compared to complex formation
within reaction mixtures containing the test compound and a mutant HGPRBMY1 or
HGPRBMY2. This comparison may be important in those cases wherein it is desirable
to identify compounds that disrupt interactions of mutant but not normal HGPRBMY1
20 or HGPRBMY2.

The assay for compounds that interfere with the interaction of the HGPRBMY1
or HGPRBMY2 and binding partners can be conducted in a heterogeneous or
homogeneous format. Heterogeneous assays involve anchoring either the HGPRBMY1
25 or HGPRBMY2 moiety product or the binding partner onto a solid phase and detecting
complexes anchored on the solid phase at the end of the reaction. In homogeneous assays,
the entire reaction is carried out in a liquid phase. In either approach, the order of addition
of reactants can be varied to obtain different information about the compounds being
tested. For example, test compounds that interfere with the interaction by competition can
30 be identified by conducting the reaction in the presence of the test substance; *i.e.*, by
adding the test substance to the reaction mixture prior to or simultaneously with the
HGPRBMY1 or HGPRBMY2 moiety and interactive binding partner. Alternatively, test
compounds that disrupt preformed complexes, *e.g.* compounds with higher binding
constants that displace one of the components from the complex, can be tested by adding
35 the test compound to the reaction mixture after complexes have been formed. The various
formats are described briefly below.

In a heterogeneous assay system, either the HGPRBMY1 or HGPRBMY2 moiety
5 or the interactive binding partner, is anchored onto a solid surface, while the
non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates
are conveniently utilized. The anchored species may be immobilized by non-covalent or
covalent attachments. Non-covalent attachment may be accomplished simply by coating
10 the solid surface with a solution of the HGPRBMY1 or HGPRBMY2 gene product or
binding partner and drying. Alternatively, an immobilized antibody specific for the
species to be anchored may be used to anchor the species to the solid surface. The
surfaces may be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed
15 to the coated surface with or without the test compound. After the reaction is complete,
unreacted components are removed (*e.g.*, by washing) and any complexes formed will
remain immobilized on the solid surface. The detection of complexes anchored on the
solid surface can be accomplished in a number of ways. Where the non-immobilized
species is pre-labeled, the detection of label immobilized on the surface indicates that
20 complexes were formed. Where the non-immobilized species is not pre-labeled, an
indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a
labeled antibody specific for the initially non-immobilized species (the antibody, in turn,
may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending
25 upon the order of addition of reaction components, test compounds which inhibit
complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or
absence of the test compound, the reaction products separated from unreacted
components, and complexes detected; *e.g.*, using an immobilized antibody specific for
30 one of the binding components to anchor any complexes formed in solution, and a labeled
antibody specific for the other partner to detect anchored complexes. Again, depending
upon the order of addition of reactants to the liquid phase, test compounds which inhibit
complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used.
35 In this approach, a preformed complex of the HGPRBMY1 or HGPRBMY2 moiety and
the interactive binding partner is prepared in which either the HGPRBMY1 or
HGPRBMY2 or its binding partners is labeled, but the signal generated by the label is

quenched due to formation of the complex (see, *e.g.*, U.S. Pat. No. 4,109,496 by
5 Rubenstein which utilizes this approach for immunoassays). The addition of a test
substance that competes with and displaces one of the species from the preformed
complex will result in the generation of a signal above background. In this way, test
substances which disrupt HGPRBMY1 or HGPRBMY2/intracellular binding partner
interaction can be identified.

10 In a particular embodiment, an HGPRBMY1 or HGPRBMY2 fusion can be
prepared for immobilization. For example, the HGPRBMY1 or HGPRBMY2
polypeptides or peptides, *e.g.*, corresponding to the CD, can be fused to a
glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such
15 a manner that its binding activity is maintained in the resulting fusion polypeptide. The
interactive binding partner can be purified and used to raise a monoclonal antibody, using
methods routinely practiced in the art and described above, in Section 5.3. This antibody
can be labeled with the radioactive isotope ^{125}I , for example, by methods routinely
practiced in the art. In a heterogeneous assay, *e.g.*, the GST-HGPRBMY1 or
20 HGPRBMY2 fusion polypeptide can be anchored to glutathione-agarose beads. The
interactive binding partner can then be added in the presence or absence of the test
compound in a manner that allows interaction and binding to occur. At the end of the
reaction period, unbound material can be washed away, and the labeled monoclonal
antibody can be added to the system and allowed to bind to the complexed components.
25 The interaction between the HGPRBMY1 or HGPRBMY2 gene product and the
interactive binding partner can be detected by measuring the amount of radioactivity that
remains associated with the glutathione-agarose beads. A successful inhibition of the
interaction by the test compound will result in a decrease in measured radioactivity.

30 Alternatively, the GST-HGPRBMY1 or HGPRBMY2 fusion polypeptide and the
interactive binding partner can be mixed together in liquid in the absence of the solid
glutathione-agarose beads. The test compound can be added either during or after the
species are allowed to interact. This mixture can then be added to the glutathione-agarose
beads and unbound material is washed away. Again the extent of inhibition of the
35 HGPRBMY1 or HGPRBMY2/binding partner interaction can be detected by adding the
labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed
5 using polypeptides or peptides that correspond to the binding domains of the
HGPRBMY1 or HGPRBMY2 and/or the interactive or binding partner (in cases where
the binding partner is a polypeptide), in place of one or both of the full length
polypeptides. Any number of methods routinely practiced in the art can be used to
10 identify and isolate the binding sites. These methods include, but are not limited to,
mutagenesis of the gene encoding one of the polypeptides and screening for disruption
of binding in a co-immunoprecipitation assay. compensating mutations in the gene
encoding the second species in the complex can then be selected. Sequence analysis of
the genes encoding the respective polypeptides will reveal the mutations that correspond
15 to the region of the polypeptide involved in interactive binding.

Alternatively, one polypeptide can be anchored to a solid surface using methods
described above, and allowed to interact with and bind to its labeled binding partner,
which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short,
20 labeled peptide comprising the binding domain may remain associated with the solid
material, which can be isolated and identified by amino acid sequencing. Also, once the
gene coding for the intracellular binding partner is obtained, short gene segments can be
engineered to express polypeptides or peptides of the invention, which can then be tested
for binding activity and purified or synthesized.

For example, and not by way of limitation, an HGPRBMY1 or HGPRBMY2
25 gene product can be anchored to a solid material by making a GST-HGPRBMY1 or
HGPRBMY2 fusion polypeptide and allowing it to bind to glutathione agarose beads.
The interactive binding partner can be labeled with a radioactive isotope, such as ^{35}S , and
cleaved with a proteolytic enzyme such as trypsin. Cleavage products can then be added
30 to the anchored GST-HGPRBMY1 or HGPRBMY2 fusion polypeptide and allowed to
bind. After washing away unbound peptides, labeled bound material, representing the
intracellular binding partner binding domain, can be eluted, purified, and analyzed for
amino acid sequence by well-known methods. Peptides so identified can be produced
synthetically or fused to appropriate facilitative polypeptides using recombinant DNA
35 technology.

5.5.4. Assays for Identification of Compounds that Ameliorate Immune Disorders

Compounds, including but not limited to binding compounds identified via assay techniques such as those described, above, in Sections 5.5.1 through 5.5.3, can be tested for the ability to ameliorate immune related disorder symptoms, including immunodeficiency. The assays described above can identify compounds which affect HGPRBMY1 activity (*e.g.*, compounds that bind to the HGPRBMY1, inhibit binding of the natural ligand, and either activate signal transduction (agonists) or block activation (antagonists), and compounds that bind to the natural ligand of the HGPRBMY1 and neutralize ligand activity); or compounds that affect HGPRBMY1 gene activity (by affecting HGPRBMY1 gene expression, including molecules, *e.g.*, polypeptides or small organic molecules, that affect or interfere with splicing events so that expression of the full length or the truncated form of the HGPRBMY1 can be modulated). However, it should be noted that the assays described can also identify compounds that modulate HGPRBMY1 signal transduction (*e.g.*, compounds which affect downstream signaling events, such as inhibitors or enhancers of tyrosine kinase or phosphatase activities which participate in transducing the signal activated by agonist or antagonist binding to the HGPRBMY1). The identification and use of such compounds which affect another step in the HGPRBMY1 signal transduction pathway in which the HGPRBMY1 gene and/or HGPRBMY1 gene product is involved and, by affecting this same pathway may modulate the effect of HGPRBMY1 on the development of immune disorders are within the scope of the invention. Such compounds can be used as part of a therapeutic method for the treatment of immune disorders.

The invention features cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate immune related disorder symptoms. Such cell-based assay systems can also be used as a standard to assay for purity and potency of the natural ligand, agonist or antagonist, including recombinantly or synthetically produced agonist or antagonist and agonist or antagonist mutants.

Cell-based systems can be used to identify compounds which may act to ameliorate immune related disorder symptoms. Such cell systems can include, for example, recombinant or non-recombinant cells, such as cell lines, which express the HGPRBMY1 gene. For example bone marrow or spleen cells, or cell lines derived from bone marrow or spleen can be used. In addition, expression host cells (*e.g.*, COS cells,

CHO cells, fibroblasts) genetically engineered to express a functional HGPRBMY1 and
5 to respond to activation by the natural agonist or antagonist ligand, *e.g.*, as measured by
a chemical or phenotypic change, induction of another host cell gene, change in ion flux
(*e.g.*, Ca^{++}), tyrosine phosphorylation of host cell polypeptides, etc., can be used as an end
point in the assay.

10 In utilizing such cell systems, cells may be exposed to a compound suspected of
exhibiting an ability to ameliorate immune related disorder symptoms, at a sufficient
concentration and for a time sufficient to elicit such an amelioration of immune related
disorder symptoms in the exposed cells. After exposure, the cells can be assayed to
measure alterations in the expression of the HGPRBMY1 gene, *e.g.*, by assaying cell
15 lysates for HGPRBMY1 mRNA transcripts (*e.g.*, by Northern analysis) or for
HGPRBMY1 polypeptide expressed in the cell; compounds which regulate or modulate
expression of the HGPRBMY1 gene are good candidates as therapeutics. Alternatively,
the cells are examined to determine whether one or more immune related disorder-like
cellular phenotypes has been altered to resemble a more normal or more wild type,
20 non-immune related disorder phenotype, or a phenotype more likely to produce a lower
incidence or severity of disorder symptoms.

Still further, the expression and/or activity of components of the signal
transduction pathway of which HGPRBMY1 is a part, or the activity of the HGPRBMY1
25 signal transduction pathway itself can be assayed. For example, after exposure, the cell
lysates can be assayed for the presence of tyrosine phosphorylation of host cell
polypeptides, as compared to lysates derived from unexposed control cells. The ability
of a test compound to inhibit tyrosine phosphorylation of host cell polypeptides in these
assay systems indicates that the test compound inhibits signal transduction initiated by
30 HGPRBMY1 activation. The cell lysates can be readily assayed using a Western blot
format; *i.e.*, the host cell polypeptides are resolved by gel electrophoresis, transferred and
probed using a anti-phosphorylated amino acid detection antibody (*e.g.*, an
anti-phosphotyrosine antibody labeled with a signal generating compound, such as
radiolabel, fluor, enzyme, etc.) (See, *e.g.*, Glenney et al., 1988, J. Immunol. Methods
35 109:277-285; Frackelton et al., 1983, Mol. Cell. Biol. 3:1343-1352). Alternatively, an
ELISA format could be used in which a particular host cell polypeptide involved in the
HGPRBMY1 signal transduction pathway is immobilized using an anchoring antibody

specific for the target host cell polypeptide, and the presence or absence of
5 phosphorylated amino acid residues, for example on tyrosine, on the immobilized host
cell polypeptide is detected using a labeled anti-phosphotyrosine antibody (See, King et
al., 1993, Life Sciences 53:1465-1472). In yet another approach, ion flux, such as calcium
ion flux, can be measured as an end point for HGPRBMY1 stimulated signal
transduction.

10 In addition, animal-based immune related disorder systems may for example be
used to identify compounds capable of ameliorating immune related disorder-like
symptoms. Such animal models may be used as test substrates for the identification of
drugs, pharmaceuticals, therapies and interventions which may be effective in treating
15 such disorders. For example, animal models may be exposed to a compound, suspected
of exhibiting an ability to ameliorate immune related disorder symptoms, at a sufficient
concentration and for a time sufficient to elicit such an amelioration of immune related
disorder symptoms in the exposed animals. The response of the animals to the exposure
may be monitored by assessing the reversal of disorders associated with immune
20 disorders such as immunodeficiency. With regard to intervention, any treatments which
reverse any aspect of immune related disorder-like symptoms should be considered as
candidates for human immune related disorder therapeutic intervention. Dosages of test
agents may be determined by deriving dose-response curves, as discussed in Section
25 5.7.1, below.

5.5.4b. Assays for Identification of Compounds that Ameliorate Cardiovascular Disorders

Compounds, including but not limited to binding compounds identified via assay
30 techniques such as those described, above, in Sections 5.5.1 through 5.5.3, can be tested
for the ability to ameliorate cardiovascular disorder symptoms, including congestive heart
failure. The assays described above can identify compounds which affect HGPRBMY2
activity (*e.g.*, compounds that bind to the HGPRBMY2, inhibit binding of the natural
ligand, and either activate signal transduction (agonists) or block activation (antagonists),
35 and compounds that bind to the natural ligand of the HGPRBMY2 and neutralize ligand
activity); or compounds that affect HGPRBMY2 gene activity (by affecting HGPRBMY2
gene expression, including molecules, *e.g.*, polypeptides or small organic molecules, that

affect or interfere with splicing events so that expression of the full length or the truncated form of the HGPRBMY2 can be modulated). However, it should be noted that the assays described can also identify compounds that modulate HGPRBMY2 signal transduction (*e.g.*, compounds which affect downstream signalling events, such as inhibitors or enhancers of tyrosine kinase or phosphatase activities which participate in transducing the signal activated by agonist or antagonist binding to the HGPRBMY2). The identification and use of such compounds which affect another step in the HGPRBMY2 signal transduction pathway in which the HGPRBMY2 gene and/or HGPRBMY2 gene product is involved and, by affecting this same pathway may modulate the effect of HGPRBMY2 on the development of cardiovascular disorders are within the scope of the invention. Such compounds can be used as part of a therapeutic method for the treatment of cardiovascular disorders.

The invention encompasses cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate cardiovascular disorder symptoms. Such cell-based assay systems can also be used as a standard to assay for purity and potency of the natural ligand, agonist or antagonist, including recombinantly or synthetically produced agonist or antagonist and agonist or antagonist mutants.

Cell-based systems can be used to identify compounds which may act to ameliorate cardiovascular disorder symptoms. Such cell systems can include, for example, recombinant or non-recombinant cells, such as cell lines, which express the HGPRBMY2 gene. For example heart cells, or cell lines derived from heart can be used. In addition, expression host cells (*e.g.*, COS cells, CHO cells, fibroblasts) genetically engineered to express a functional HGPRBMY2 and to respond to activation by the natural agonist or antagonist ligand, *e.g.*, as measured by a chemical or phenotypic change, induction of another host cell gene, change in ion flux (*e.g.*, Ca^{++}), tyrosine phosphorylation of host cell polypeptides, etc., can be used as an end point in the assay.

In utilizing such cell systems, cells may be exposed to a compound suspected of exhibiting an ability to ameliorate cardiovascular disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration of cardiovascular disorder symptoms in the exposed cells. After exposure, the cells can be assayed to measure alterations in the expression of the HGPRBMY2 gene, *e.g.*, by assaying cell

lysates for HGPRBMY2 mRNA transcripts (*e.g.*, by Northern analysis) or for
5 HGPRBMY2 polypeptide expressed in the cell; compounds which regulate or modulate
expression of the HGPRBMY2 gene are good candidates as therapeutics. Alternatively,
the cells are examined to determine whether one or more cardiovascular disorder-like
cellular phenotypes has been altered to resemble a more normal or more wild type,
10 non-cardiovascular disorder phenotype, or a phenotype more likely to produce a lower
incidence or severity of disorder symptoms. Still further, the expression and/or activity
of components of the signal transduction pathway of which HGPRBMY2 is a part, or the
activity of the HGPRBMY2 signal transduction pathway itself can be assayed. For
example, after exposure, the cell lysates can be assayed for the presence of tyrosine
15 phosphorylation of host cell polypeptides, as compared to lysates derived from unexposed
control cells. The ability of a test compound to inhibit tyrosine phosphorylation of host
cell polypeptides in these assay systems indicates that the test compound inhibits signal
transduction initiated by HGPRBMY2 activation. The cell lysates can be readily assayed
using a Western blot format; *i.e.*, the host cell polypeptides are resolved by gel
20 electrophoresis, transferred and probed using a anti-phosphotyrosine detection antibody
(*e.g.*, an anti-phosphotyrosine antibody labeled with a signal generating compound, such
as radiolabel, fluor, enzyme, etc.) (See, *e.g.*, Glenney et al., 1988, J. Immunol. Methods
109:277-285; Frackelton et al., 1983, Mol. Cell. Biol. 3:1343-1352). Alternatively, an
25 ELISA format could be used in which a particular host cell polypeptide involved in the
HGPRBMY2 signal transduction pathway is immobilized using an anchoring antibody
specific for the target host cell polypeptide, and the presence or absence of
phosphotyrosine on the immobilized host cell polypeptide is detected using a labeled
anti-phosphotyrosine antibody. (See, King et al., 1993, Life Sciences 53:1465-1472). In
30 yet another approach, ion flux, such as calcium ion flux, can be measured as an end point
for HGPRBMY2 stimulated signal transduction.

In addition, animal-based cardiovascular disorder systems may for example be
used to identify compounds capable of ameliorating cardiovascular disorder-like
symptoms. Such animal models may be used as test substrates for the identification of
35 drugs, pharmaceuticals, therapies and interventions which may be effective in treating
such disorders. For example, animal models may be exposed to a compound, suspected
of exhibiting an ability to ameliorate cardiovascular disorder symptoms, at a sufficient

concentration and for a time sufficient to elicit such an amelioration of cardiovascular disorder symptoms in the exposed animals. The response of the animals to the exposure may be monitored by assessing the reversal of disorders associated with cardiovascular disorders such as congestive heart failure. With regard to intervention, any treatments which reverse any aspect of cardiovascular disorder-like symptoms should be considered as candidates for human cardiovascular disorder therapeutic intervention. Dosages of test agents may be determined by deriving dose-response curves, as discussed in Section 5.7.1, below.

5.6. The Treatment of Immune related, Including Immune Disorders

The invention features methods and compositions for modifying immune related disorders and treating immune disorders, including but not limited to immunodeficiency. Because a loss of normal HGPRBMY1 gene product function results in the development of immune related disease, an increase in HGPRBMY1 gene product activity, or activation of the HGPRBMY1 pathway (*e.g.*, downstream activation) would facilitate progress towards a normal immune related state in individuals exhibiting a deficient level of HGPRBMY1 gene expression and/or HGPRBMY1 activity.

Alternatively, symptoms of certain immune disorders such as, for example, immunodeficiency may be ameliorated by modulating (increasing or decreasing) the level of HGPRBMY1 gene expression, and/or HGPRBMY1 gene activity, and/or modulating activity of the HGPRBMY1 pathway (*e.g.*, by targeting downstream signaling events). Different approaches are discussed below.

HGPRBMY1 is expressed in bone marrow, spleen and thymus tissues, thus HGPRBMY1 nucleic acids, polypeptides, and modulators thereof can be used to modulate the proliferation, development, differentiation, and/or function of immune cells, *e.g.* B-cells, dendritic cells, natural killer cells and monocytes, and/or immune function. HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized to modulate immune-related processes, *e.g.*, the host immune response by, for example, modulating the formation of and/or binding to immune complexes, detection and defense against surface antigens and bacteria, and immune surveillance for rapid removal or pathogens.

HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized
5 to modulate or treat immune disorders that include, but are not limited to, immune
proliferative disorders (*e.g.*, carcinoma, lymphoma, *e.g.*, follicular lymphoma), and
disorders associated with fighting pathogenic infections, (*e.g.*, bacterial (*e.g.*, chlamydia)
infection, parasitic infection, and viral infection (*e.g.*, HSV or HIV infection)), and
10 pathogenic disorders (*e.g.*, immunodeficiency disorders, such as HIV), autoimmune
disorders, such as arthritis, multiple sclerosis, Grave's disease, or Hashimoto's disease,
T cell disorders (*e.g.*, AIDS) and inflammatory disorders, such as septicemia, cerebral
malaria, inflammatory bowel disease, arthritis (*e.g.*, rheumatoid arthritis, osteoarthritis),
and allergic inflammatory disorders (*e.g.*, asthma, psoriasis), apoptotic disorders (*e.g.*,
15 rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus),
cytotoxic disorders, septic shock, and cachexia.

HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized
to regulate immune activation to suppress rejection of a grafted organ or grafted tissue
in a graft recipient (*e.g.*, to prevent allograft rejection).

20 HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized
to modulate immune activation. For example, antagonists to HGPRBMY1 action, such
as peptides, antibodies or small molecules that decrease or block HGPRBMY1 activity,
e.g., binding to extracellular matrix components, *e.g.*, integrins, or that prevent
25 HGPRBMY1 signaling, can be used as immune system activation blockers. In another
example, agonists that mimic or partially mimic HGPRBMY1 activity, such as peptides,
antibodies or small molecules, can be used to induce immune system activation.
Antibodies may activate or inhibit the cell adhesion, proliferation and activation, and may
help in treating infection, autoimmunity, inflammation, and cancer by affecting these
30 cellular processes.

HGPRBMY1 nucleic acids, polypeptides and modulators thereof can also be
utilized to modulate intercellular signaling in the immune system, *e.g.*, modulate
intercellular signal transduction in immune stimulation or suppression and modulate
immune cell membrane adhesion to extra-cellular matrix components.

35 As HGPRBMY1 is expressed in bone marrow, HGPRBMY1 nucleic acids,
polypeptides, and modulators thereof can be used to diagnose disorders associated with
cells in the bone marrow and/or modulate the proliferation, differentiation, and/or

function of cells that appear in the bone marrow, *e.g.*, stem cells (*e.g.*, hematopoietic stem
5 cells), and blood cells, *e.g.*, erythrocytes, platelets, and leukocytes. Thus HGPRBMY1
nucleic acids, polypeptides, and modulators thereof can be used to treat bone marrow,
blood, and hematopoietic associated diseases and disorders, *e.g.*, acute myeloid leukemia,
hemophilia, leukemia, anemia (*e.g.*, sickle cell anemia), and thalassemia.

As HGPRBMY1 is expressed in the thymus, HGPRBMY1 nucleic acids,
10 polypeptides, and modulators thereof can be used to diagnose thymus associated
disorders. HGPRBMY1 nucleic acids, polypeptides, and modulators thereof can also be
used modulate the proliferation, development, differentiation, maturation and/or function
of thymocytes, *e.g.*, modulate development and maturation of T-lymphocytes.
15 HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized to
modulate immune-related processes such as the ability to modulate host immune
response by, *e.g.*, modulating the formation of and/or binding to immune complexes, and
modulating the positive and negative selection of thymocytes. Such HGPRBMY1
compositions and modulators thereof can be utilized, *e.g.*, to ameliorate incidence of any
20 symptoms associated with disorders that involve such immune-related processes,
including, but not limited to infection and autoimmune disorders (*e.g.*, insulin-dependent
mellitus, multiple sclerosis, systemic lupus, erythematosus, sjogren's syndrome,
autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, Grave's disease,
25 idiopathic thrombocytopenia purpura, rheumatoid arthritis, and scleroderma).
HGPRBMY1 nucleic acids, polypeptides and modulators thereof can also be utilized to
treat viral infections, inflammatory immune disorders and immune-related cancers
including but not limited to, leukemia (*e.g.*, acute leukemia, chronic leukemia, Hodgkin's
disease non-Hodgkin's lymphoma, and multiple myeloma).

30 HGPRBMY1 has structural homology with the receptor for the serine protease,
thrombin. As such HGPRBMY1 nucleic acids, polypeptides and modulators thereof can
be utilized to modulate activities, processes or disorders associated with protease activity,
e.g., serine protease activity. For example, HGPRBMY1 nucleic acids, polypeptides or
35 modulators thereof can be used to modulate serine protease activities, such as those
activities associated with such serine proteases (or, where appropriate, human
homologues thereof), *e.g.*, adipsin (complement factor D), acrosin, thrombin,
plasminogen, protein C, cathepsin G, chymotrypsin, complement components and

signaling, cytotoxic cell proteases, duodenase I, elastases 1, 2, 3A, 3B and medullasin,
5 enterokinase, hepatocyte growth factor activator, hepsin, kallikreins, gamma-renin,
prostate specific antigen, mast cell proteases, myeloblastin, Alzheimer's plaque-related
proteases, tryptases, ancrod, batroxobin, cerastobin, flavoxobin, apolipoprotein, blood
fluke cercarial protease, Drosophila trypsin like protease (*e.g.*, alpha, easter, and snake
10 locus), Drosophila protease stubble, or major mite fecal antigen.

10 HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be used to
modulate processes and/or diseases involved with serine protease response activity. For
example, such processes and/or diseases can include, but are not limited to cellular
activation, cellular proliferation, motility and differentiation, the alternative complement
15 pathway, *e.g.*, disturbances of the complement regulation system, such as complement
regulator deficiencies, which include, for example, hereditary angioedema (an allergic
disorder) and proxysmal nocturnal hemoglobinuria (the presence of hemoglobin in the
urine), modulate body weight or body weight disorders, *e.g.*, obesity or cachexia,
systemic energy balance and diabetes.

20 In addition, assays can be developed to measure the biological activity of
polypeptides or peptides of the invention. In particular, HGPRBMY1 or modulators
thereof, biological activities include, *e.g.*, (1) the ability to modulate development,
differentiation, proliferation and/or activity of immune cells (*e.g.*, leukocytes and
25 macrophages), endothelial cells and smooth muscle cells; (2) the ability to modulate the
host immune response; (3) the ability to modulate intracellular signaling cascades (*e.g.*,
signal transduction cascades); (4) the ability to modulate the development of organs,
tissues and/or cells of the embryo and/or fetus; (5) the ability to modulate cell-cell
interactions and/or cell-extracellular matrix interactions; (6) the ability to modulate
30 atherosclerosis, *e.g.*, the initiation and progression of atherosclerosis; (7) the ability to
modulate atherogenesis; (8) the ability to modulate inflammatory functions *e.g.*, by
modulating leukocyte adhesion to extracellular matrix and/or endothelial cells; (9) the
ability to bind and phagocytose cells, *e.g.*, aged and apoptotic cells; (10) the ability to
remove debris, *e.g.*, apoptotic cells, from blood vessel walls; (11) the ability to modulate,
35 *e.g.*, inhibit, the expression of molecules, *e.g.*, adhesion molecules (*e.g.*, leukocyte
adhesion molecules) and growth factors (*e.g.*, smooth-muscle growth factors); (12) the
ability to alter, *e.g.*, increase, expression in response to stimuli, *e.g.*, TNF, shear stress,

and pathophysiological stimuli relevant to disorders (*e.g.*, atherosclerosis and
5 inflammation); and (13) the ability to form, *e.g.*, stabilize, promote, facilitate, inhibit, or
disrupt, cell to cell and cell to blood product interaction, *e.g.*, between leukocytes and
platelets or leukocytes and vascular endothelial cells.

10 **5.6b. The Treatment of Cardiovascular, Including Cardiovascular Disorders**

The invention encompasses methods and compositions for modifying
cardiovascular and treating cardiovascular disorders, including but not limited to
congestive heart failure. Because a loss of normal HGPRBMY2 gene product function
results in the development of cardiovascular disease, an increase in HGPRBMY2 gene
15 product activity, or activation of the HGPRBMY2 pathway (*e.g.*, downstream activation)
would facilitate progress towards a normal cardiovascular state in individuals exhibiting
a deficient level of HGPRBMY2 gene expression and/or HGPRBMY2 activity.
Alternatively, symptoms of certain cardiovascular disorders such as, for example,
congestive heart failure may be ameliorated by modulating (increasing or decreasing) the
20 level of HGPRBMY2 gene expression, and/or HGPRBMY2 gene activity, and/or
modulating activity of the HGPRBMY2 pathway (*e.g.*, by targeting downstream
signalling events). Different approaches are discussed below.

25 **5.6c. The Treatment of Neurological Disorders and Diseases**

Nervous system diseases, disorders, and/or conditions, which can be treated,
prevented, and/or diagnosed with the compositions of the invention (*e.g.*, HGPRBMY2
polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited
to, nervous system injuries, and diseases, disorders, and/or conditions which result in
30 either a disconnection of axons, a diminution or degeneration of neurons, or
demyelination. Nervous system lesions which may be treated, prevented, and/or
diagnosed in a patient (including human and non-human mammalian patients) according
to the invention, include but are not limited to, the following lesions of either the central
(including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in
35 which a lack of oxygen in a portion of the nervous system results in neuronal injury or
death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2)
traumatic lesions, including lesions caused by physical injury or associated with surgery,

for example, lesions which sever a portion of the nervous system, or compression
5 injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or
injured by malignant tissue which is either a nervous system associated malignancy or a
malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a
portion of the nervous system is destroyed or injured as a result of infection, for example,
10 by an abscess or associated with infection by human immunodeficiency virus, herpes
zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5)
degenerative lesions, in which a portion of the nervous system is destroyed or injured as
a result of a degenerative process including but not limited to degeneration associated
with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic
15 lateral sclerosis (ALS); (6) lesions associated with nutritional diseases, disorders, and/or
conditions, in which a portion of the nervous system is destroyed or injured by a
nutritional disorder or disorder of metabolism including but not limited to, vitamin B12
deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia,
Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and
20 alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic
diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy),
systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic
substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated
25 lesions in which a portion of the nervous system is destroyed or injured by a
demyelinating disease including, but not limited to, multiple sclerosis, human
immunodeficiency virus-associated myelopathy, transverse myelopathy or various
etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the HGPRBMY2 polypeptides, polynucleotides, or
30 agonists or antagonists of the invention are used to protect neural cells from the damaging
effects of cerebral hypoxia. According to this embodiment, the compositions of the
invention are used to treat, prevent, and/or diagnose neural cell injury associated with
cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides,
or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose
35 neural cell injury associated with cerebral ischemia. In another aspect of this
embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the
invention are used to treat, prevent, and/or diagnose neural cell injury associated with

cerebral infarction. In another aspect of this embodiment, the polypeptides,
5 polynucleotides, or agonists or antagonists of the invention are used to treat, prevent,
and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect
of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the
invention are used to treat, prevent, and/or diagnose neural cell injury associated with a
heart attack.

10 The HGPRBMY2 compositions of the invention which are useful for treating or
preventing a nervous system disorder may be selected by testing for biological activity
in promoting the survival or differentiation of neurons. For example, and not by way of
limitation, compositions of the invention which elicit any of the following effects may
15 be useful according to the invention: (1) increased survival time of neurons in culture; (2)
increased sprouting of neurons in culture or in vivo; (3) increased production of a neuron-
associated molecule in culture or in vivo, e.g., choline acetyltransferase or
acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron
dysfunction in vivo. Such effects may be measured by any method known in the art. In
20 preferred, non-limiting embodiments, increased survival of neurons may routinely be
measured using a method set forth herein or otherwise known in the art, such as, for
example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990));
increased sprouting of neurons may be detected by methods known in the art, such as, for
example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or
25 Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-
associated molecules may be measured by bioassay, enzymatic assay, antibody binding,
Northern blot assay, etc., using techniques known in the art and depending on the
molecule to be measured; and motor neuron dysfunction may be measured by assessing
30 the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron
conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that
may be treated, prevented, and/or diagnosed according to the invention include, but are
not limited to, diseases, disorders, and/or conditions such as infarction, infection,
35 exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may
affect motor neurons as well as other components of the nervous system, as well as
diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic

lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy,
5 progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

10 **5.6.1. Modulation of HGPRBMY1 Expression or Activity**

Any method which neutralizes an agonist or antagonist or modulates expression of the HGPRBMY1 gene (*e.g.*, by either activating or decreasing transcription or translation) can be used to prevent HGPRBMY1 immune disorders.

15 For example, the administration of soluble peptides, polypeptides, fusion polypeptides, or antibodies (including anti-idiotypic antibodies) that bind to a circulating agonist or antagonist, the natural ligand for the HGPRBMY1, can be used to prevent or treat immune disorders. To this end, peptides corresponding to the ECD of HGPRBMY1, soluble deletion mutants of HGPRBMY1 (*e.g.*, Δ TM-HGPRBMY1 mutants), or either
20 of these HGPRBMY1 domains or mutants fused to another polypeptide (*e.g.*, an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic antibodies or Fab fragments of antiidiotypic antibodies that mimic the HGPRBMY1 ECD and neutralize agonists or antagonists can be used (see Section 5.3, *supra*). Such HGPRBMY1 polypeptides, peptides, fusion polypeptides, anti-idiotypic antibodies or Fabs are administered to a
25 subject in amounts sufficient to neutralize agonist or antagonist and to prevent or treat immune disorders.

Fusion of the HGPRBMY1, the HGPRBMY1 ECD or the Δ TMHGPRBMY1 to an IgFc polypeptide should not only increase the stability of the preparation, but will
30 increase the half-life and activity of the HGPRBMY1-Ig fusion polypeptide *in vivo*. The Fc region of the Ig portion of the fusion polypeptide may be further modified to reduce immunoglobulin effector function. In an alternative embodiment for neutralizing circulating agonist or antagonist, cells that are genetically engineered to express such soluble or secreted forms of HGPRBMY1 may be administered to a patient, whereupon
35 they will serve as "bioreactors" *in vivo* to provide a continuous supply of the agonist or antagonist neutralizing polypeptide. Such cells may be obtained from the patient or an MHC compatible donor and can include, but are not limited to fibroblasts, blood cells

(*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are
5 genetically engineered *in vitro* using recombinant DNA techniques to introduce the
coding sequence for the HGPRBMY1 ECD, Δ TMHGPRBMY1, or for HGPRBMY1-Ig
fusion polypeptide (*e.g.*, HGPRBMY1-, ECD- or Δ TMHGPRBMY1-IgFc fusion
polypeptides) into the cells, etc. by transduction (using viral vectors, and preferably
10 vectors that integrate the transgene into the cell genome) or transfection procedures,
including but not limited to the use of plasmids, cosmids, YACs, electroporation,
liposomes, etc. The HGPRBMY1 coding sequence can be placed under the control of a
strong constitutive or inducible promoter or promoter/enhancer to achieve expression and
secretion of the HGPRBMY1 peptide or fusion polypeptide. The engineered cells which
15 express and secrete the desired HGPRBMY1 product can be introduced into the patient
systemically, *e.g.*, in the circulation, intraperitoneally, at the heart. Alternatively, the cells
can be incorporated into a matrix and implanted in the body, *e.g.*, genetically engineered
fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial
cells can be implanted as part of a vascular graft (See, for example, Anderson et al. U.S.
20 Pat. No. 5,399,349; and Mulligan & Wilson, U.S. Pat. No. 5,460,959 each of which is
incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous cells, they can be
administered using well known techniques which prevent the development of a host
25 immune response against the introduced cells. For example, the cells may be introduced
in an encapsulated form which, while allowing for an exchange of components with the
immediate extracellular environment, does not allow the introduced cells to be
recognized by the host immune system.

In an alternate embodiment, immune disorder therapy can be designed to reduce
30 the level of endogenous HGPRBMY1 gene expression, *e.g.*, using antisense or ribozyme
approaches to inhibit or prevent translation of HGPRBMY1 mRNA transcripts; triple
helix approaches to inhibit transcription of the HGPRBMY1 gene; or targeted
homologous recombination to inactivate or "knock out" the HGPRBMY1 gene or its
endogenous promoter. Alternatively, the antisense, ribozyme or DNA constructs
35 described herein could be administered directly to the site containing the target cells; *e.g.*,
the bone marrow or spleen.

Antisense approaches involve the design of oligonucleotides (either DNA or
5 RNA) that are complementary to HGPRBMY1 mRNA. The antisense oligonucleotides
will bind to the complementary HGPRBMY1 mRNA transcripts and prevent translation.
Absolute complementarity, although preferred, is not required. A sequence
"complementary" to a portion of an RNA, as referred to herein, means a sequence having
10 sufficient complementarity to be able to hybridize with the RNA, forming a stable
duplex; in the case of double-stranded antisense nucleic acids, a single strand of the
duplex DNA may thus be tested, or triplex formation may be assayed. The ability to
hybridize will depend on both the degree of complementarity and the length of the
antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base
15 mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the
case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use
of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the
5' untranslated sequence up to and including the AUG initiation codon, should work most
20 efficiently at inhibiting translation. However, sequences complementary to the 3'
untranslated sequences of mRNAs have recently shown to be effective at inhibiting
translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335.
Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding
25 regions of the HGPRBMY1 shown in SEQ ID NO:1, could be used in an antisense
approach to inhibit translation of endogenous HGPRBMY1 mRNA. Oligonucleotides
complementary to the 5' untranslated region of the mRNA should include the
complement of the AUG start codon. Antisense oligonucleotides complementary to
mRNA coding regions are less efficient inhibitors of translation but could be used in
30 accordance with the invention. Whether designed to hybridize to the 5', 3'- or coding
region of HGPRBMY1 mRNA, antisense nucleic acids should be at least six nucleotides
in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in
length. In specific aspects the oligonucleotide is at least nucleotides, at least 17
nucleotides, at least 25 nucleotides or at least 50 nucleotides.

35 Regardless of the choice of target sequence, it is preferred that *in vitro* studies are
first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene
expression. It is preferred that these studies utilize controls that distinguish between

antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or polypeptide with that of an internal control RNA or polypeptide. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published Dec. 15, 1988), hybridization-triggered cleavage agents (See, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (See, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, β -D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, β -D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil,

5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc. While antisense nucleic acids complementary to the HGPRBMY1 coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred. For example, antisense oligonucleotides having the following sequences can be utilized in accordance with the invention:

- a) 5'-CATCCGCCTTATTACAT-3' (SEQ ID NO:28) which is complementary to nucleotides -14 to +3 as shown in SEQ ID NO:1;
- b) 5'-CATCCGCCTTATTACATCTTTT-3' (SEQ ID NO:29) which is complementary to nucleotides -20 to +3 in SEQ ID NO:1.

The antisense molecules should be delivered to cells which express the HGPRBMY1 *in vivo*, *e.g.*, the bone marrow or spleen. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can

be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs. Therefore a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous HGPRBMY1 transcripts and thereby prevent translation of the HGPRBMY1 mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site; *e.g.*, the bone marrow or spleen. Alternatively, viral vectors can be used which selectively infect the desired tissue; (*e.g.*, for bone marrow or spleen, herpesvirus vectors may be used or alternatively, in dividing bone marrow cells retroviruses may be used), in which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules-designed to catalytically cleave HGPRBMY1 mRNA transcripts can also be used to prevent translation of HGPRBMY1mRNA and expression

of HGPRBMY1. (See, *e.g.*, PCT International Publication WO90/11364, published Oct. 4, 1990; Sarver et al., 1990, Science 247:1222-1225). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy HGPRBMY1 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, Nature, 334:585-591. There are hundreds of potential hammerhead ribozyme cleavage sites within the nucleic acid sequence of human HGPRBMY1 cDNA. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the HGPRBMY1 mRNA; *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. For example, hammerhead ribozymes can be utilized in accordance with the invention.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena Thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, Science, 224:574-578; Zaug and Cech, 1986, Science, 231:470-475; Zaug, et al., 1986, Nature, 324:429-433; published International patent-application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, Cell, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention features those Cech-type ribozymes which target eight base-pair active site sequences that are present in HGPRBMY1.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.* for improved stability, targeting, etc.) and should be delivered to cells which express the HGPRBMY1 *in vivo*, *e.g.*, bone marrow or spleen. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous HGPRBMY1 messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous HGPRBMY1 gene expression can also be reduced by inactivating the HGPRBMY1 gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies et al., 1985, Nature 317:230-234; Thomas & Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989 Cell 5:313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional HGPRBMY1, or unrelated sequences, which are flanked by DNA homologous to the endogenous HGPRBMY1 gene locus can be used with or without a selectable marker and/or a negative selectable marker, to transfect cells that express HGPRBMY1 *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the HGPRBMY1 gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive HGPRBMY1 (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors, *e.g.*, herpes virus vectors for delivery to tissue; *e.g.*, bone marrow or spleen.

Alternatively, endogenous HGPRBMY1 gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the HGPRBMY1 gene (*i.e.*, the HGPRBMY1 promoter and/or enhancers) to form triple helical structures that prevent transcription of the HGPRBMY1 gene in target cells in the body. (See generally, Helene, C. 1991, Anticancer Drug Des., 6(6):569-84; Helene, C., et al., 1992, Ann. N.Y. Acad. Sci., 660:27-36; and Maher, L. J., 1992, Bioassays 14(12):807-15).

In yet another embodiment of the invention, the activity of HGPRBMY1 can be reduced using a "dominant negative" approach to prevent or treat immune disorders. To this end, constructs which encode defective HGPRBMY1 can be used in gene therapy approaches to diminish the activity of the HGPRBMY1 in appropriate target cells. For example, nucleic acid sequences that direct host cell expression of HGPRBMY1 in which the CD is deleted or mutated can be introduced into cells in the bone marrow or spleen (either by *in vivo* or *ex vivo* gene therapy methods described above). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject's endogenous HGPRBMY1 gene in the bone marrow or

spleen. The engineered cells will express non-functional receptors (*i.e.*, an anchored receptor that is capable of binding its natural ligand, but incapable of signal transduction). Such engineered cells present in the bone marrow or spleen should demonstrate a diminished response to the endogenous agonist or antagonist ligand, resulting in immune disorders.

With respect to an increase in the level of normal HGPRBMY1 gene expression and/or HGPRBMY1 gene product activity, HGPRBMY1 nucleic acid sequences can be utilized for the treatment of immune disorders, including immunodeficiency. Where the cause of immunodeficiency is a defective HGPRBMY1, treatment can be administered, for example, in the form of gene replacement therapy.

In another embodiment, the expression characteristics of an endogenous gene (*e.g.*, HGPRBMY1 genes) within a cell, cell line or microorganism may be modified by inserting a DNA regulatory element heterologous to the endogenous gene of interest into the genome of a cell, stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous gene (*e.g.*, HGPRBMY1 genes) and controls, modulates or activates. For example, endogenous HGPRBMY1 genes which are normally "transcriptionally silent", *i.e.*, a HGPRBMY1 genes which is normally not expressed, or are expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, transcriptionally silent, endogenous HGPRBMY1 genes may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with and activates expression of endogenous HGPRBMY1 genes, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991; Skoultchi U.S. Patent No. 5,981,214; Treco *et al* U.S. Patent No. 5,968,502 and PCT publication No. WO 94/12650, published June 9, 1994. Alternatively, non-targeted *e.g.*, non-homologous recombination techniques which are well-known to those of skill

in the art and described, *e.g.*, in PCT publication No. WO 99/15650, published April 1, 1999, may be used.

Specifically, one or more copies of a normal HGPRBMY1 gene or a portion of the HGPRBMY1 gene that directs the production of an HGPRBMY1 gene product exhibiting normal function, may be inserted into the appropriate cells within a patient or animal subject, using vectors which include, but are not limited to adenovirus, adeno-associated virus, retrovirus and herpes virus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

Because the HGPRBMY1 gene is expressed in the bone marrow, spleen and thymus, such gene replacement therapy techniques should be capable of delivering HGPRBMY1 gene sequences to these cell types within patients. Thus, the techniques for delivery of the HGPRBMY1 gene sequences should be designed to readily involve direct administration of such HGPRBMY1 gene sequences to the site of the cells in which the HGPRBMY1 gene sequences are to be expressed. Alternatively, targeted homologous recombination can be utilized to correct the defective endogenous HGPRBMY1 gene in the appropriate tissue; *e.g.*, bone marrow or spleen cells (particularly B-cells). In animals, targeted homologous recombination can be used to correct the defect in ES cells in order to generate offspring with a corrected trait.

Additional methods which may be utilized to increase the overall level of HGPRBMY1 gene expression and/or HGPRBMY1 activity include the introduction of appropriate HGPRBMY1-expressing cells, preferably autologous cells, into a patient at positions and in numbers which are sufficient to ameliorate the symptoms of immune disorders, including immunodeficiency. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase the overall level of HGPRBMY1 gene expression in a patient are normal cells, preferably bone marrow or spleen cells, cells which express the HGPRBMY1 gene. The cells can be administered at the anatomical site in the body, or as part of a tissue graft located at a different site in the body. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, et al., U.S. Pat. No. 5,399,349; Mulligan & Wilson, U.S. Pat. No. 5,460,959.

Finally, compounds, identified in the assays described above, that stimulate or enhance the signal transduced by activated HGPRBMY1, *e.g.*, by activating downstream

signaling polypeptides in the HGPRBMY1 cascade and thereby by-passing the defective HGPRBMY1, can be used to ameliorate immune related disease. The formulation and mode of administration will depend upon the physico-chemical properties of the compound.

5.6.1b. Modulation of HGPRBMY2 Expression or HGPRBMY2 Activity to Prevent Heart Failure

Any method which neutralizes an agonist or antagonist or modulates expression of the HGPRBMY2 gene (either transcription or translation) can be used to prevent heart failure or heart disease. Such approaches can be used to treat any cardiovascular disorder.

For example, the administration of soluble peptides, polypeptides, fusion polypeptides, or antibodies that bind to the natural ligand for the HGPRBMY2, can be used to prevent or treat heart disease. To this end, peptides corresponding to the ECD of HGPRBMY2, soluble deletion mutants of HGPRBMY2 (*e.g.*, Δ TM-HGPRBMY2 mutants), or either of these HGPRBMY2 domains or mutants fused to another polypeptide (*e.g.*, an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic antibodies or fragments thereof that mimic the HGPRBMY2 ECD and neutralize agonists or antagonists can be used (see Section 5.3, *supra*). Such HGPRBMY2 polypeptides, peptides, fusion polypeptides, and/or antibodies are administered to a subject in amounts sufficient to bind the ligand and to prevent or treat heart disease.

Fusion of the HGPRBMY2, the HGPRBMY2-ECD to an IgFc polypeptide should not only increase the stability of the preparation, but will increase the half-life and activity of the HGPRBMY2-Ig fusion polypeptide *in vivo*. The Fc region of the Ig portion of the fusion polypeptide may be further modified to reduce immunoglobulin effector function. In an alternative embodiment for neutralizing circulating agonist or antagonist, cells that are genetically engineered to express such soluble or secreted forms of HGPRBMY2 may be administered to a patient to provide a continuous supply of the agonist or antagonist neutralizing polypeptide. Such cells may be obtained from the patient or an MHC compatible donor and can include, but are not limited to fibroblasts, blood cells (*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence for the HGPRBMY2 ECD, Δ TMHGPRBMY2, or for HGPRBMY2-Ig

fusion polypeptide (*e.g.*, HGPRBMY2-, ECD- or Δ TMHGPRBMY2-IgFc fusion polypeptides) into the cells, etc. by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including but not limited to the use of plasmids, cosmids, YACs, electroporation, liposomes, etc.

The HGPRBMY2 coding sequence can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression and secretion of the HGPRBMY2 peptide or fusion polypeptide. The engineered cells which express and secrete the desired HGPRBMY2 product can be introduced into the patient systemically, *e.g.*, in the circulation, intraperitoneally, at the heart. Alternatively, the cells can be incorporated into a matrix and implanted in the body, *e.g.*, genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a vascular graft. (See, for example, Anderson et al. 5,399,349; and Mulligan & Wilson, 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

In an alternate embodiment, heart failure therapy can be designed to reduce the level of endogenous HGPRBMY2 gene expression, *e.g.*, using antisense or ribozyme approaches to inhibit or prevent translation of HGPRBMY2 mRNA transcripts; triple helix approaches to inhibit transcription of the HGPRBMY2 gene; or targeted homologous recombination to inactivate or "knock out" the HGPRBMY2 gene or its endogenous promoter. Alternatively, the antisense, ribozyme or DNA constructs described herein could be administered directly to the site containing the target cells; *e.g.*, the heart.

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to HGPRBMY2 mRNA. The antisense oligonucleotides will bind to the complementary HGPRBMY2 mRNA transcripts and prevent translation.

Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of the HGPRBMY2 shown in SEQ ID NO:13, could be used in an antisense approach to inhibit translation of endogenous HGPRBMY2 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of HGPRBMY2 mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or polypeptide with that of an internal control RNA or polypeptide. Additionally, it is envisioned that results

obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published Dec. 15, 1988), hybridization-triggered cleavage agents. (See, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, β -D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, β -D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. The antisense oligonucleotide may also comprise at least one

modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleic acids complementary to the HGPRBMY2 coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred. For example, antisense oligonucleotides having the following sequences can be utilized in accordance with the invention:

- a) 5'-CATGCGGGGCAGCGAGG-3' (SEQ ID NO:30) which is complementary to nucleotides -14 to +3 as shown in SEQ ID NO:13;
- b) 5'-CATGCGGGGCAGCGAGGGCTTCGG-3' (SEQ ID NO:31) which is complementary to nucleotides -20 to +3 in SEQ ID NO:13.

The antisense molecules should be delivered to cells which express HGPRBMY2 *in vivo*, *e.g.*, the heart. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*,

antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

Alternatively, an antisense nucleic acid is delivered via a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous HGPRBMY2 transcripts and thereby prevent translation of the HGPRBMY2 mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art.

Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site; *e.g.*, the heart. Alternatively, viral vectors can be used which selectively infect the desired tissue; (*e.g.*, for heart, herpesvirus vectors may be used), in which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules-designed to catalytically cleave HGPRBMY2 mRNA transcripts can also be used to prevent translation of HGPRBMY2mRNA and expression of HGPRBMY2. (See, *e.g.*, PCT International Publication WO90/11364, published Oct. 4, 1990; Sarver et al., 1990, Science 247:1222-1225). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy HGPRBMY2 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave

mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, *Nature*, 334:585-591. There are hundreds of potential hammerhead ribozyme cleavage sites within the nucleic acid sequence of human HGPRBMY2 cDNA. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the HGPRBMY2 mRNA; *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. For example, hammerhead ribozymes can be utilized in accordance with the invention.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena Thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, *Science*, 224:574-578; Zaug and Cech, 1986, *Science*, 231:470-475; Zaug, et al., 1986, *Nature*, 324:429-433; published International patent-application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, *Cell*, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in HGPRBMY2.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.* for improved stability, targeting, etc.) and should be delivered to cells which express the HGPRBMY2 *in vivo*, *e.g.*, heart. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous HGPRBMY2 messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous HGPRBMY2 gene expression can also be reduced by inactivating or "knocking out" the HGPRBMY2 gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies et al., 1985, *Nature* 317:230-234; Thomas & Capecchi, 1987, *Cell* 51:503-512; Thompson et al., 1989 *Cell* 5:313-321; each of which is

incorporated by reference herein in its entirety). For example, a mutant, non-functional HGPRBMY2 flanked by DNA homologous to the endogenous HGPRBMY2 gene locus, coding or regulatory, can be used with or without a selectable marker and/or a negative selectable marker to transfect cells that express HGPRBMY2 *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the HGPRBMY2 gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive HGPRBMY2 (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors, *e.g.*, herpes virus vectors for delivery to tissue; *e.g.*, heart.

Alternatively, endogenous HGPRBMY2 gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the HGPRBMY2 gene (*i.e.*, the HGPRBMY2 promoter and/or enhancers) to form triple helical structures that prevent transcription of the HGPRBMY2 gene in target cells in the body. (See generally, Helene, C. 1991, *Anticancer Drug Des.*, 6(6):569-84; Helene, C., et al., 1992, *Ann. N.Y. Acad. Sci.*, 660:27-36; and Maher, L. J., 1992, *Bioassays* 14(12):807-15).

In yet another embodiment of the invention, the activity of HGPRBMY2 can be reduced using a "dominant negative" approach to prevent or treat heart failure. To this end, constructs which encode defective HGPRBMY2 can be used in gene therapy approaches to diminish the activity of the HGPRBMY2 in appropriate target cells. For example, nucleic acid sequences that direct host cell expression of HGPRBMY2 in which the CD is deleted or mutated can be introduced into cells in the heart (either by *in vivo* or *ex vivo* gene therapy methods described above). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject's endogenous HGPRBMY2 gene in the heart. The engineered cells will express non-functional receptors (*i.e.*, an anchored receptor that is capable of binding its natural ligand, but incapable of signal transduction). Such engineered cells present in the heart should demonstrate a diminished response to the endogenous agonist or antagonist ligand, resulting in heart failure.

With respect to an increase in the level of normal HGPRBMY2 gene expression and/or HGPRBMY2 gene product activity, HGPRBMY2 nucleic acid sequences can be utilized for the treatment of cardiovascular disorders, including congestive heart failure. Where the cause of congestive heart failure is a defective HGPRBMY2, treatment can be administered, for example, in the form of gene replacement therapy.

In another embodiment, the expression characteristics of an endogenous gene (*e.g.*, HGPRBMY2 genes) within a cell, cell line or microorganism may be modified by inserting a DNA regulatory element heterologous to the endogenous gene of interest into the genome of a cell, stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous gene (*e.g.*, HGPRBMY2 genes) and controls, modulates or activates. For example, endogenous HGPRBMY2 genes which are normally "transcriptionally silent", *i.e.*, a HGPRBMY2 genes which is normally not expressed, or are expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, transcriptionally silent, endogenous HGPRBMY2 genes may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with and activates expression of endogenous HGPRBMY2 genes, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991; Skoultchi U.S. Patent No. 5,981,214; Treco *et al* U.S. Patent No. 5,968,502 and PCT publication No. WO 94/12650, published June 9, 1994. Alternatively, non-targeted *e.g.*, non-homologous recombination techniques which are well-known to those of skill in the art and described, *e.g.*, in PCT publication No. WO 99/15650, published April 1, 1999, may be used.

Specifically, one or more copies of a normal HGPRBMY2 gene or a portion of the HGPRBMY2 gene that directs the production of an HGPRBMY2 gene product exhibiting normal function, may be inserted into the appropriate cells within a patient or animal subject, using vectors which include, but are not limited to adenovirus,

adeno-associated virus, retrovirus and herpes virus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

Because the HGPRBMY2 gene is expressed in the heart and thymus, such gene replacement therapy techniques should be capable of delivering HGPRBMY2 gene sequences to these cell types within patients. Thus, the techniques for delivery of the HGPRBMY2 gene sequences should be designed to readily involve direct administration of such HGPRBMY2 gene sequences to the site of the cells in which the HGPRBMY2 gene sequences are to be expressed. Alternatively, targeted homologous recombination can be utilized to correct the defective endogenous HGPRBMY2 gene in the appropriate tissue; *e.g.*, heart. In animals, targeted homologous recombination can be used to correct the defect in ES cells in order to generate offspring with a corrected trait.

Additional methods which may be utilized to increase the overall level of HGPRBMY2 gene expression and/or HGPRBMY2 activity include the introduction of appropriate HGPRBMY2-expressing cells, preferably autologous cells, into a patient at positions and in numbers which are sufficient to ameliorate the symptoms of cardiovascular disorders, including congestive heart failure. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase the overall level of HGPRBMY2 gene expression in a patient are normal cells, preferably heart cells, cells which express the HGPRBMY2 gene. The cells can be administered at the anatomical site in the body, or as part of a tissue graft located at a different site in the body. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, et al., 5,399,349; Mulligan & Wilson, U.S. Pat. No. 5,460,959.

Finally, compounds, identified in the assays described above, that stimulate or enhance the signal transduced by activated HGPRBMY2, *e.g.*, by activating downstream signalling polypeptides in the HGPRBMY2 cascade and thereby by-passing the defective HGPRBMY2, can be used to ameliorate cardiovascular disease. The formulation and mode of administration will depend upon the physico-chemical properties of the compound.

5.7. Pharmaceutical Preparations and Methods of Administration

The compounds that are determined to affect HGPRBMY1 gene expression or HGPRBMY1 activity can be administered to a patient at therapeutically effective doses to treat or ameliorate bone marrow or spleen disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of immune disorders.

The compounds that are determined to affect HGPRBMY2 gene expression or HGPRBMY2 activity can be administered to a patient at therapeutically effective doses to treat or ameliorate heart disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of cardiovascular or neural disorders.

5.7.1. Effective Dose

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred.

While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test

compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

5.7.2. Formulations and Use

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion.

Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

References

Draetta, G. Mammalian G1 cyclins. *Curr. Opin. Cell Biol.* 6, 842-846 (1994).

Schafer, KA. The cell cycle: a review. *Vet Pathol* 1998 35, 461-478 (1998).

Medema, R. H.; Kops, G. J. P. L.; Bos, J. L.; Burgering, B. M. T. AFX-like forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27(kip1). *Nature* 40, 782-787 (2000).

Lee MH, Yang HY. Negative regulators of cyclin-dependent kinases and their roles in cancers. *Cell Mol Life Sci.* 2001 58, 1907-1922 (2001).

Desdouets C. and Brechot C. p27: a pleiotropic regulator of cellular phenotype and a target for cell cycle dysregulation in cancer. *Pathol Biol (Paris)* 48, 203-210 (2000).

Sgambato A, Cittadini A, Faraglia B, Weinstein IB. Multiple functions of p27(Kip1) and its alterations in tumor cells: a review. *J Cell Physiol.* 183, 18-27 (2000).

EXAMPLES

Example 1 – HGPRBMY1 Bioinformatics Analysis

G-protein coupled receptor sequences were used as a probe to search the Incyte and public domain EST databases. All the G-protein coupled receptor sequences available at the GPCRdb GPCR Database (<http://www.gpcr.org/7tm>) were used as queries. The search program used was gapped BLAST (Altschul, et al., 1997, *Nucleic Acids Res* 25:3389-3402). The top EST hits from the BLAST results were searched back against the non-redundant polypeptide and patent sequence databases. From this analysis, ESTs encoding a potential novel GPCRs were identified based on sequence homology. The public domain EST (ATCC® CloneID: 145375) was selected as potential novel GPCR candidate, HGPRBMY1 for subsequent analysis.

This EST was sequenced over its full length and was shown to contain a coding region bearing distinctive characteristics of a G-protein coupled receptor (GPCR). More

specifically, the complete polypeptide sequence of HGPRBMY1 was analyzed for potential transmembrane domains. The TMPRED program was used for transmembrane prediction (K Hofmann and W Stoffel, 1993, Biol. Chem. Hoppe-Seyler 347:166). The program predicted seven transmembrane domains and the predicted domains match with the predicted transmembrane domains of related GPCRs at the sequence level. Based on sequence, structure and known GPCR signature sequences, the orphan polypeptide, HGPRBMY1, is likely a novel human GPCR.

Example 2 - Cloning of the Novel Human GPCR HGPRBMY1

A PCR primer pair, designed from the DNA sequence of ATCC® clone was used to amplify a piece of DNA from the same clone in which the antisense strand of the amplified fragment was biotinylated on the 3' end. This biotinylated piece of double stranded DNA was denatured and incubated with a mixture of single-stranded covalently closed circular cDNA libraries which contain DNA corresponding to the sense strand.

Hybrids between the biotinylated DNA and the circular cDNA were captured on streptavidin magnetic beads. Upon thermal release of the cDNA from the biotinylated DNA, the single stranded cDNA was converted into double strands using a primer homologous to a sequence on the cDNA cloning vector. The double stranded cDNA was introduced into *E. coli* by electroporation and the resulting colonies were screen by PCR, using the original primer pair, to identify the proper cDNA.

Example 3 - Expression profiling of novel human GPCR, HGPRBMY1

A PCR primer was designed from the ATCC® clone and was used to measure the steady state levels of mRNA by quantitative PCR. The sequence of the primer pair was as follows:

5'-GATCCCCGTCGGTCATCTT-3' (SEQ ID NO:3)

5'-GGTCACCACGTTGCAAAGC-3' (SEQ ID NO:4)

Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA

in each sample and these data were used for normalization of the data obtained with the primer pair for this gene. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested and the data is presented in Figure 5.

Transcripts corresponding to the orphan GPCR, HGPRBMY1, are expressed highly in bone marrow and spleen, and to a lesser extent in the thymus.

Example 4 - Complementary Polynucleotides And Association of HGPRBMY1 To Cell Cycle and Apoptosis Regulation

Antisense molecules or nucleic acid sequences complementary to the HGPRBMY1 protein-encoding sequence, or any part thereof, is used to decrease or to inhibit the expression of naturally occurring HGPRBMY1. Although the use of antisense or complementary oligonucleotides comprising about 15 to 35 base-pairs is described, essentially the same procedure is used with smaller or larger nucleic acid sequence fragments. An oligonucleotide based on the coding sequence of HGPRBMY1 protein, as shown in Figure 1, or as depicted in SEQ ID NO:1, for example, is used to inhibit expression of naturally occurring HGPRBMY1. The complementary oligonucleotide is typically designed from the most unique 5' sequence and is used either to inhibit transcription by preventing promoter binding to the coding sequence, or to inhibit translation by preventing the ribosome from binding to the HGPRBMY1 protein-encoding transcript, among others. However, other regions may also be targeted.

Using an appropriate portion of the signal and 5' sequence of SEQ ID NO:1, an effective antisense oligonucleotide includes any of about 15-35 nucleotides spanning the region which translates into the signal or 5' coding sequence, among other regions, of the polypeptide as shown in Figure 2 (SEQ ID NO:2). Appropriate oligonucleotides are designed using OLIGO 4.06 software and the HGPRBMY1 protein coding sequence (SEQ ID NO:1). Preferred oligonucleotides are deoxynucleotide-, or chimeric deoxynucleotide/ribonucleotide-based and are provided below. The oligonucleotides were synthesized using chemistry essentially as described in U.S. Patent No. 5,849,902; which is hereby incorporated herein by reference in its entirety.

ID#	Sequence
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- 15214 ACAGGAUGCAACGCUUAAGUCGACG (SEQ ID NO:5)
- 15215 AGAUUUGGAAAGGCAACACGCUGGC (SEQ ID NO:6)
- 15216 CUUGAGGACGUCGAAGCAGGUGAUG (SEQ ID NO:7)
- 15217 GCCUCCUCCGUGCGCAACAGCUUGA (SEQ ID NO:8)
- 15218 CUGAUACAGGUCAUGGUGAGGAUGC (SEQ ID NO:9)

The HGPRBMY1 polypeptide has been shown to be involved in the regulation of mammalian cell cycle pathways. Subjecting cells with an effective amount of a pool of all five of the above antisense oligonucleotides (SEQ ID NO:5 thru 9) resulted in a significant increase in p27 expression/activity providing convincing evidence that HGPRBMY1 at least regulates the activity and/or expression of p27 either directly, or indirectly. Moreover, the results suggest that HGPRBMY1 is involved in the negative regulation of p27 activity and/or expression, either directly or indirectly. The p27 assay used is described below and was based upon the analysis of p27 activity as a downstream marker for proliferative signal transduction events.

Moreover, the HGPRBMY1 polypeptide has also been shown to be involved in the regulation of mammalian NF- κ B and apoptosis pathways. Subjecting cells with an effective amount of a pool of all five of the above antisense oligonucleotides (SEQ ID NO:5 thru 9) resulted in a significant increase in I κ B α expression/activity providing convincing evidence that HGPRBMY1 at least regulates the activity and/or expression of I κ B α either directly, or indirectly. Moreover, the results suggest that HGPRBMY1 is involved in the negative regulation of NF- κ B/I κ B α activity and/or expression, either directly or indirectly. The I κ B α assay used is described below and was based upon the analysis of I κ B α activity as a downstream marker for proliferative signal transduction events.

Based upon the regulation of p27 and I κ B, antagonists directed against HGPRBMY1 would be useful for upregulating P27 and I κ B, which would be beneficial to cancer patients by stopping proliferation and inducing apoptosis of a cell comprising a tumor.

Transfection of post-quiescent A549 cells With AntiSense Oligonucleotides.

Materials needed:

- A549 cells maintained in DMEM with high glucose (Gibco-BRL) supplemented with 10% Fetal Bovine Serum, 2mM L-Glutamine, and 1X penicillin/streptomycin.
- Opti-MEM (Gibco-BRL)
- Lipofectamine 2000 (Invitrogen)
- Antisense oligomers (Sequitur)
- Polystyrene tubes.
- Tissue culture treated plates.

Quiescent cells were prepared as follows:

Day 0: 300,000 A549 cells were seeded in a T75 tissue culture flask in 10 ml of A549 media, and incubated at 37°C, 5% CO₂ in a humidified incubator for 48 hours.

Day 2: The T75 flasks were rocked to remove any loosely adherent cells, and the A549 growth media removed and replenished with 10 ml of fresh A549 media. The cells were cultured for six days without changing the media to create a quiescent cell population.

Day 8: Quiescent cells were plated in multi-well format and transfected with antisense oligonucleotides.

A549 cells were transfected according to the following:

1. Trypsinize T75 flask containing quiescent population of A549 cells.
2. Count the cells and seed 24-well plates with 60K quiescent A549 cells per well.
3. Allow the cells to adhere to the tissue culture plate (approximately 4 hours).
4. Transfect the cells with antisense and control oligonucleotides according to the following:

- a. A 10X stock of lipofectamine 2000 (10 ug/ml is 10X) was prepared, and diluted lipid was allowed to stand at RT for 15 minutes.
Stock solution of lipofectamine 2000 was 1 mg/ml.
10 X solution for transfection was 10 ug/ml.
To prepare 10X solution, dilute 10 ul of lipofectamine 2000 stock per 1 ml of Opti-MEM (serum free media).
- b. A 10X stock of each oligomer was prepared to be used in the transfection.
Stock solutions of oligomers were at 100 uM in 20 mM HEPES, pH 7.5.
10X concentration of oligomer was 0.25 uM.
To prepare the 10X solutions, dilute 2.5 ul of oligomer per 1 ml of Opti-MEM.
- c. Equal volumes of the 10X lipofectamine 2000 stock and the 10X oligomer solutions were mixed well, and incubated for 15 minutes at RT to allow complexation of the oligomer and lipid. The resulting mixture was 5X.
- d. After the 15 minute complexation, 4 volumes of full growth media was added to the oligomer/lipid complexes (solution was 1X).
- e. The media was aspirated from the cells, and 0.5 ml of the 1X oligomer/lipid complexes added to each well.
- f. The cells were incubated for 16-24 hours at 37°C in a humidified CO₂ incubator.
- g. Cell pellets were harvested for RNA isolation and TaqMan analysis of downstream marker genes.

TaqMan Reactions – p27 Reactions

Quantitative RT-PCR analysis was performed on total RNA preps that had been treated with DNaseI or poly A selected RNA. The Dnase treatment may be performed using methods known in the art, though preferably using a Qiagen RNeasy kit to purify the RNA samples, wherein DNase I treatment is performed on the column.

Briefly, a master mix of reagents was prepared according to the following table:

Dnase I Treatment

<u>Reagent</u>	<u>Per rxn (in uL)</u>
10x Buffer	2.5
Dnase I (1 unit/ul @ 1 unit per ug 2 sample)	
DEPC H ₂ O	0.5
RNA sample @ 0.1 ug/ul (2-3 ug total)	20
Total	25

Next, 5 ul of master mix was aliquoted per well of a 96-well PCR reaction plate (PE part # N801-0560). RNA samples were adjusted to 0.1 ug/ul with DEPC treated H₂O (if necessary), and 20 ul was added to the aliquoted master mix for a final reaction volume of 25 ul.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and briefly spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient

The plates were incubated at 37°C for 30 mins. Then, an equal volume of 0.1mM EDTA in 10mM Tris was added to each well, and heat inactivated at 70°C for 5 min. The plates were stored at -80°C upon completion.

RT reaction

A master mix of reagents was prepared according to the following table:

RT reaction

	<u>I</u>	<u>No RT</u>
<u>Reagent</u>	<u>rxn (in ul)</u>	<u>rxn (in ul)</u>
10x RT buffer	5	2.5
MgCl ₂	11	5.5

DNTP mixture	10	5
Random Hexamers	2.5	1.25
Rnase inhibitors	1.25	0.625
RT enzyme	1.25	-
Total RNA 500ng (100ng 19.0 max no RT)		10.125 max
DEPC H ₂ O	-	-
Total	50uL	25uL

Samples were adjusted to a concentration so that 500ng of RNA was added to each RT rx'n (100ng for the no RT). A maximum of 19 ul can be added to the RT rx'n mixture (10.125 ul for the no RT.) Any remaining volume up to the maximum values was filled with DEPC treated H₂O, so that the total reaction volume was 50 ul (RT) or 25 ul (no RT).

On a 96-well PCR reaction plate (PE part # N801-0560), 37.5 ul of master mix was aliquoted (22.5 ul of no RT master mix), and the RNA sample added for a total reaction volume of 50ul (25 ul, no RT). Control samples were loaded into two or even three different wells in order to have enough template for generation of a standard curve.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and spin briefly in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

For the RT-PCR reaction, the following thermal profile was used:

- 25°C for 10 min
- 48°C for 30 min
- 95°C for 5 min
- 4°C hold (for 1 hour)
- Store plate @-20°C or lower upon completion.

TaqMan reaction (Template comes from RT plate.)

A master mix was prepared according to the following table:

TaqMan reaction (per well)

<u>Reagent</u>	<u>Per Rx'n (in ul)</u>
TaqMan Master Mix	4.17
100 uM Probe (SEQ ID NO:12)	.025
100 uM Forward primer (SEQ ID NO:10)	.05
100 uM Reverse primer (SEQ ID NO:11)	.05
Template	-
DEPC H ₂ O	18.21
Total	22.5

The primers used for the RT-PCR reaction is as follows:

P27 primer and probes:

Forward Primer: CCCGGTGGACCACGAA (SEQ ID NO:10)

Reverse Primer: GGCTCGCCTCTTCCATGTC (SEQ ID NO:11)

TaqMan Probe: AACCCGGGACTTGGAGAAGCACTGC (SEQ ID NO:12)

TaqMan Reactions – Ikb Reactions

Quantitative RT-PCR analysis was performed on total RNA preps that had been treated with DNaseI or poly A selected RNA. The Dnase treatment may be performed using methods known in the art, though preferably using a Qiagen RNeasy kit to purify the RNA samples, wherein DNase I treatment is performed on the column.

Briefly, a master mix of reagents was prepared according to the following table:

Dnase I Treatment

<u>Reagent</u>	<u>Per r'xn (in uL)</u>
10x Buffer	2.5
Dnase I (1 unit/ul @ 1 unit per ug 2 sample)	
DEPC H ₂ O	0.5
RNA sample @ 0.1 ug/ul (2-3 ug total)	20
Total	25

Next, 5 ul of master mix was aliquoted per well of a 96-well PCR reaction plate (PE part # N801-0560). RNA samples were adjusted to 0.1 ug/ul with DEPC treated H₂O (if necessary), and 20 ul was added to the aliquoted master mix for a final reaction volume of 25 ul.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and briefly spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient

The plates were incubated at 37°C for 30 mins. Then, an equal volume of 0.1mM EDTA in 10mM Tris was added to each well, and heat inactivated at 70°C for 5 min. The plates were stored at -80°C upon completion.

RT reaction

A master mix of reagents was prepared according to the following table:

RT reaction

	<u>RT</u>	<u>No RT</u>
<u>Reagent</u>	<u>μl (in ul)</u>	<u>μl (in ul)</u>
10x RT buffer	5	2.5
MgCl ₂	11	5.5
DNTP mixture	10	5
Random Hexamers	2.5	1.25
Rnase inhibitors	1.25	0.625
RT enzyme	1.25	-
Total RNA 500ng (100ng 19.0 max no RT)		10.125 max
DEPC H ₂ O	-	-
Total	50uL	25uL

Samples were adjusted to a concentration so that 500ng of RNA was added to each RT rx'n (100ng for the no RT). A maximum of 19 ul can be added to the RT rx'n mixture (10.125 ul for the no RT.) Any remaining volume up to the maximum values was filled with DEPC treated H₂O, so that the total reaction volume was 50 ul (RT) or 25 ul (no RT).

On a 96-well PCR reaction plate (PE part # N801-0560), 37.5 ul of master mix was aliquoted (22.5 ul of no RT master mix), and the RNA sample added for a total reaction volume of 50ul (25 ul, no RT). Control samples were loaded into two or even three different wells in order to have enough template for generation of a standard curve.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and spin briefly in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

For the RT-PCR reaction, the following thermal profile was used:

- 25°C for 10 min
- 48°C for 30 min
- 95°C for 5 min

- 4°C hold (for 1 hour)
- Store plate @ -20°C or lower upon completion.

TaqMan reaction (Template comes from RT plate.)

A master mix was prepared according to the following table:

TaqMan reaction (per well)

<u>Reagent</u>	<u>Per Rx'n (in ul)</u>
TaqMan Master Mix	4.17
100 uM Probe	.025
(SEQ ID NO:15)	
100 uM	.05
Forward primer (SEQ ID NO:13)	
100 uM	.05
Reverse primer (SEQ ID NO:14)	
Template	-
DEPC H ₂ O	18.21
Total	22.5

The primers used for the RT-PCR reaction is as follows:

IkB primer and probes:

Forward Primer: GAGGATGAGGAGAGCTATGACACA (SEQ ID NO:13)

Reverse Primer: CCCTTTGCACTCATAACGTCAG (SEQ ID NO:14)

TaqMan Probe: AAACACACAGTCATCATAGGGCAGCTCGT (SEQ ID NO:15)

Using a Gilson P-10 repeat pipetter, 22.5 ul of master mix was aliquouted per well of a 96-well optical plate. Then, using P-10 pipetter, 2.5 ul of sample was added to individual wells. Generally, RT samples are run in triplicate with each primer/probe set used, and no RT samples are run once and only with one primer/probe set, often gapdh (or other internal control).

A standard curve is then constructed and loaded onto the plate. The curve has five points plus one no template control (NTC, =DEPC treated H₂O). The curve was made with a high point of 50 ng of sample (twice the amount of RNA in unknowns), and successive samples of 25, 10, 5, and 1 ng. The curve was made from a control sample(s) (see above).

The wells were capped using optical strip well caps (PE part # N801-0935), placed in a plate, and spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

Plates were loaded onto a PE 5700 sequence detector making sure the plate is aligned properly with the notch in the upper right hand corner. The lid was tightened down and run using the 5700 and 5700 quantitation program and the SYBR probe using the following thermal profile:

- 50°C for 2 min
- 95°C for 10 min
- and the following for 40 cycles:
 - 95°C for 15 sec
 - 60°C for 1 min
- Change the reaction volume to 25ul.

Once the reaction was complete, a manual threshold of around 0.1 was set to minimize the background signal. Additional information relative to operation of the

GeneAmp 5700 machine may be found in reference to the following manuals: "GeneAmp 5700 Sequence Detection System Operator Training CD"; and the "User's Manual for 5700 Sequence Detection System"; available from Perkin-Elmer and hereby incorporated by reference herein in their entirety.

Example 5 – HGPRBMY2 Bioinformatics Analysis

G-protein coupled receptor sequences were used as a probe to search the Incyte and public domain EST databases. All the G-protein coupled receptor sequences available at the GPCRdb GPCR Database (<http://www.gpcr.org/7tm>) were used as queries. The search program used was gapped BLAST (Altschul, et al., 1997, Nucleic Acids Res 25:3389-3402). The top EST hits from the BLAST results were searched back against the non-redundant polypeptide and patent sequence databases. From this analysis, ESTs encoding a potential novel GPCRs were identified based on sequence homology. The public domain EST (ATCC CloneID: 3293096) was selected as potential novel GPCR candidate, HGPRBMY2 for subsequent analysis.

This EST was sequenced and the full-length clone of this GPCR was obtained using the EST sequence information. The complete polypeptide sequence of HGPRBMY2 was analyzed for potential transmembrane domains. The TMPRED program was used for transmembrane prediction (K Hofmann and W Stoffel, 1993, Biol. Chem. Hoppe-Seyler 347:166). The program predicted seven transmembrane domains and the predicted domains match with the predicted transmembrane domains of related GPCRs at the sequence level. Based on sequence, structure and known GPCR signature sequences, the orphan polypeptide, HGPRBMY2, is likely a novel human GPCR.

Example 6 - Cloning of the Novel Human GPCR HGPRBMY2

A PCR primer pair, designed from the DNA sequence of ATCC clone was used to amplify a piece of DNA from the same clone in which the antisense strand of the amplified fragment was biotinylated on the 3' end. This biotinylated piece of double stranded DNA was denatured and incubated with a mixture of single-stranded covalently closed circular cDNA libraries which contain DNA corresponding to the sense strand.

Hybrids between the biotinylated DNA and the circular cDNA were captured on streptavidin magnetic beads. Upon thermal release of the cDNA from the biotinylated

DNA, the single stranded cDNA was converted into double strands using a primer homologous to a sequence on the cDNA cloning vector. The double stranded cDNA was introduced into *E. coli* by electroporation and the resulting colonies were screen by PCR, using the original primer pair, to identify the proper cDNA.

Example 7 - Expression profiling of novel human GPCR, HGPRBMY2

A PCR primer was designed from the ATCC clone and was used to measure the steady state levels of mRNA by quantitative PCR. The sequence of the primer pair was as follows:

5'-TTTCTGGATCGTCAGCTTGCT- 3'(SEQ ID NO:15)

5'-ACAGGGCTGGTCCACTCTTCT-3'(SEQ ID NO:16)

Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA in each sample and these data were used for normalization of the data obtained with the primer pair for this gene. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested and the data is presented in Figure 10.

Transcripts corresponding to the orphan GPCR, HGPRBMY2, are expressed highly in heart, testes and to a lesser degree in thymus.

Example 8 – Method Of Assessing The Expression Profile Of The Novel HGPRBMY2 Polypeptides Of The Present Invention Using Expanded mRNA Tissue and Cell Sources

Total RNA from tissues was isolated using the TriZol protocol (Invitrogen) and quantified by determining its absorbance at 260nm. An assessment of the 18s and 28s ribosomal RNA bands was made by denaturing gel electrophoresis to determine RNA integrity.

The specific sequence to be measured was aligned with related genes found in GenBank to identify regions of significant sequence divergence to maximize primer and

probe specificity. Gene-specific primers and probes were designed using the ABI primer
5 express software to amplify small amplicons (150 base pairs or less) to maximize the
likelihood that the primers function at 100% efficiency. All primer/probe sequences were
searched against Public Genbank databases to ensure target specificity. Primers and
probes were obtained from ABI.

10 For HGPRBMY2, the primer probe sequences were as follows

Forward Primer 5'- CACCAACCGAAGGGCTTTC -3' (SEQ ID NO:25)

Reverse Primer 5'- CCACATGGGTGATCCTACGAT -3' (SEQ ID NO:26)

15 TaqMan Probe 5' - ACTGCCACCAGCCAGACCACACCTA -3' (SEQ ID NO:27)

DNA contamination

To access the level of contaminating genomic DNA in the RNA, the RNA was
20 divided into 2 aliquots and one half was treated with Rnase-free Dnase (Invitrogen).
Samples from both the Dnase-treated and non-treated were then subjected to reverse
transcription reactions with (RT+) and without (RT-) the presence of reverse
transcriptase. TaqMan assays were carried out with gene-specific primers (see above) and
25 the contribution of genomic DNA to the signal detected was evaluated by comparing the
threshold cycles obtained with the RT+/RT- non-Dnase treated RNA to that on the
RT+/RT- Dnase treated RNA. The amount of signal contributed by genomic DNA in the
Dnased RT- RNA must be less than 10% of that obtained with Dnased RT+ RNA. If not
the RNA was not used in actual experiments.

30

35

5 **Reverse Transcription reaction and Sequence Detection**

100ng of Dnase-treated total RNA was annealed to 2.5 μ M of the respective gene-specific reverse primer in the presence of 5.5 mM Magnesium Chloride by heating the sample to 72°C for 2 min and then cooling to 55° C for 30 min. 1.25 U/ μ l of MuLv
10 reverse transcriptase and 500 μ M of each dNTP was added to the reaction and the tube was incubated at 37° C for 30 min. The sample was then heated to 90°C for 5 min to denature enzyme.

Quantitative sequence detection was carried out on an ABI PRISM 7700 by adding to the reverse transcribed reaction 2.5 μ M forward and reverse primers, 500 μ M
15 of each dNTP, buffer and 5U AmpliTaq Gold™. The PCR reaction was then held at 94°C for 12 min, followed by 40 cycles of 94° C for 15 sec and 60° C for 30 sec.

Data handling

20 The threshold cycle (Ct) of the lowest expressing tissue (the highest Ct value) was used as the baseline of expression and all other tissues were expressed as the relative abundance to that tissue by calculating the difference in Ct value between the baseline and the other tissues and using it as the exponent in $2^{(\Delta C_t)}$

The expanded expression profile of the HGPRBMY2 polypeptide, is provided in
25 Figure 16 and are described elsewhere herein.

Example 9 - Functional Characterization of the novel human GPCR, HGPRBMY2

The use of mammalian cell reporter assays to demonstrate functional coupling of known GPCRs (G Protein Coupled Receptors) has been well documented in the literature
30 (Gilman, 1987, Boss et al., 1996; Alam & Cook, 1990; George et al., 1997; Selbie & Hill, 1998; Rees et al., 1999). In fact, reporter assays have been successfully used for identifying novel small molecule agonists or antagonists against GPCRs as a class of drug targets (Zlokarnik et al., 1998; George et al., 1997; Boss et al., 1996; Rees et al, 2001).
35 In such reporter assays, a promoter is regulated as a direct consequence of activation of specific signal transduction cascades following agonist binding to a GPCR (Alam & Cook 1990; Selbie & Hill, 1998; Boss et al., 1996; George et al., 1997; Gilman, 1987).

A number of response element-based reporter systems have been developed that
5 enable the study of GPCR function. These include cAMP response element (CRE)-based
reporter genes for G α i/o, G α s- coupled GPCRs, Nuclear Factor Activator of
Transcription (NFAT)-based reporters for G α q/11 -coupled receptors and MAP
kinase reporter genes for use in G α i/o coupled receptors (Selbie & Hill, 1998; Boss
10 et al., 1996; George et al., 1997; Gilman, 1987; Rees et al., 2001). Transcriptional
response elements that regulate the expression of Beta-Lactamase within a CHO K1 cell
line (Cho/NFAT-CRE: Aurora Biosciences TM) (Zlokarnik et al., 1998) have been
implemented to characterize the function of the orphan HGPRBMY2 polypeptide of the
present invention. The system enables demonstration of constitutive G-protein coupling
15 to endogenous cellular signaling components upon intracellular overexpression of orphan
receptors. Overexpression has been shown to represent a physiologically relevant event.
For example, it has been shown that overexpression occurs in nature during metastatic
carcinomas, wherein defective expression of the monocyte chemotactic protein 1
receptor, CCR2, in macrophages is associated with the incidence of human ovarian
20 carcinoma (Sica, et al., 2000; Salcedo et al., 2000). Indeed, it has been shown that
overproduction of the Beta 2 Adrenergic Receptor in transgenic mice leads to constitutive
activation of the receptor signaling pathway such that these mice exhibit increased
cardiac output (Kypson et al., 1999; Dorn et al., 1999). These are only a few of the many
25 examples demonstrating constitutive activation of GPCRs whereby many of these
receptors are likely to be in the active, R*, conformation (J.Wess 1997).

Materials and Methods:

30 DNA Constructs:

The putative GPCR HGPRBMY2 cDNA was PCR amplified using PFUTM
(Stratagene). The primers used in the PCR reaction were specific to the HGPRBMY2
polynucleotide and were ordered from Gibco BRL (5 prime primer: 5'-
35 CCCAAGCTTATGCAGGCGCTTAACATTACCCCG-3' (SEQ ID NO:17), 3 prime
primer: 5'-CGGGATCCTTAATGCCACTGTCTAAAGGAAGA-3' (SEQ ID NO:18).
The following 3 prime primer was used to add a Flag-tag epitope to the HGPRBMY2
polypeptide for immunocytochemistry: 5'-

CGGGATCCTTACTTGTCTGTCGTCGTCCTTGTAGTCCATATGCCCCTGTCTAA
5 AGGAGAATTCTCAAC-3'(SEQ ID NO:19). The product from the PCR reaction was isolated from a 0.8% Agarose gel (Invitrogen) and purified using a Gel Extraction Kit TM from Qiagen.

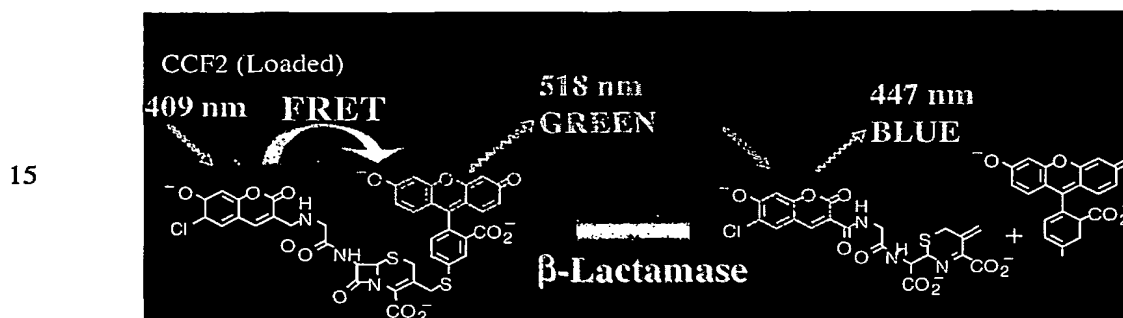
The purified product was then digested overnight along with the pcDNA3.1
10 Hygro TM mammalian expression vector from Invitrogen using the HindIII and BamHI restriction enzymes (New England Biolabs). These digested products were then purified using the Gel Extraction Kit TM from Qiagen and subsequently ligated to the pcDNA3.1 Hygro TM expression vector using a DNA molar ratio of 4 parts insert: 1 vector. All DNA modification enzymes were purchased from NEB. The ligation was incubated overnight
15 at 16 degrees Celsius, after which time, one microliter of the mix was used to transform DH5 alpha cloning efficiency competent *E. coli* TM (Gibco BRL). A detailed description of the pcDNA3.1 Hygro TM mammalian expression vector is available at the Invitrogen web site (www.invitrogen.com). The plasmid DNA from the ampicillin resistant clones were isolated using the Wizard DNA Miniprep System TM from Promega. Positive
20 clones were then confirmed and scaled up for purification using the Qiagen Maxiprep TM plasmid DNA purification kit.

Cell Line Generation:

25 The pcDNA3.1hygro vector containing the orphan HGPRBMY2 cDNA were used to transfect Cho/NFAT-CRE (Aurora Biosciences) cells using Lipofectamine 2000 TM according to the manufacturers specifications (Gibco BRL). Two days later, the cells were split 1:3 into selective media (DMEM 11056, 600 ug/ml Hygromycin, 200 ug/ml Zeocin, 10% FBS). All cell culture reagents were purchased from Gibco BRL-Invitrogen.

30 The Cho/NFAT-CRE cell lines, transiently or stably transfected with the orphan HGPRBMY2 GPCR, were analyzed using the FACS Vantage SE TM (BD), fluorescence microscopy (Nikon), and the LJI Analyst TM (Molecular Devices). In this system, changes in real-time gene expression, as a consequence of constitutive G-protein coupling of the orphan HGPRBMY2 GPCR, is examined by analyzing the fluorescence
35 emission of the transformed cells at 447nm and 518nm. The changes in gene expression can be visualized using Beta-Lactamase as a reporter, that, when induced by the appropriate signaling cascade, hydrolyzes an intracellularly loaded, membrane-permeant

ester substrate (CCF2/AMTM Aurora Biosciences; Zlokarnik, et al., 1998). The
 5 CCF2/AMTM substrate is a 7-hydroxycoumarin cephalosporin with a fluorescein attached
 through a stable thioether linkage. Induced expression of the Beta-Lactamase enzyme is
 readily apparent since each enzyme molecule produced is capable of changing the
 fluorescence of many CCF2/AMTM substrate molecules. A schematic of this cell based
 10 system is shown below.



20

In summary, CCF2/AMTM is a membrane permeant, intracellularly-trapped,
 fluorescent substrate with a cephalosporin core that links a 7-hydroxycoumarin to a
 fluorescein. For the intact molecule, excitation of the coumarin at 409 nm results in
 Fluorescence Resonance Energy Transfer (FRET) to the fluorescein which emits green
 25 light at 518 nm. Production of active Beta-Lactamase results in cleavage of the Beta-
 Lactam ring, leading to disruption of FRET, and excitation of the coumarin only - thus
 giving rise to blue fluorescent emission at 447 nm.

Fluorescent emissions were detected using a Nikon-TE300 microscope equipped
 30 with an excitation filter (D405/10X-25), dichroic reflector (430DCLP), and a barrier filter
 for dual DAPI/FITC (510nm) to visually capture changes in Beta-Lactamase expression.
 The FACS Vantage SE is equipped with a Coherent Enterprise II Argon Laser and a
 Coherent 302C Krypton laser. In flow cytometry, UV excitation at 351-364 nm from the
 Argon Laser or violet excitation at 407 nm from the Krypton laser are used. The optical
 35 filters on the FACS Vantage SE are HQ460/50m and HQ535/40m bandpass separated by
 a 490 dichroic mirror.

Prior to analyzing the fluorescent emissions from the cell lines as described

above, the cells were loaded with the CCF2/AM substrate. A 6X CCF2/AM loading
5 buffer was prepared whereby 1mM CCF2/AM (Aurora Biosciences) was dissolved in
100% DMSO (Sigma). 12 ul of this stock solution was added to 60 ul of 100mg/ml
Pluronic F127 (Sigma) in DMSO containing 0.1% Acetic Acid (Sigma). This solution
was added while vortexing to 1 mL of Sort Buffer (PBS minus calcium and magnesium-
10 Gibco-25 mM HEPES-Gibco- pH 7.4, 0.1% BSA). Cells were placed in serum-free
media and the 6X CCF2/AM was added to a final concentration of 1X. The cells were
then loaded at room temperature for one to two hours, and then subjected to fluorescent
emission analysis as described herein. Additional details relative to the cell loading
methods and/or instrument settings may be found by reference to the following
15 publications: see Zlokarnik, et al., 1998; Whitney et al., 1998; and BD Biosciences, 1999.

Immunocytochemistry:

The cell lines transfected and selected for expression of Flag-epitope tagged
orphan GPCRs were analyzed by immunocytochemistry. The cells were plated at
20 1×10^3 in each well of a glass slide (VWR). The cells were rinsed with PBS followed
by acid fixation for 30 minutes at room temperature using a mixture of 5% Glacial Acetic
Acid / 90% ETOH. The cells were then blocked in 2% BSA and 0.1% Triton in PBS,
incubated for 2 h at room temperature or overnight at 4°C. A monoclonal anti-Flag FITC
25 antibody was diluted at 1:50 in blocking solution and incubated with the cells for 2 h at
room temperature. Cells were then washed three times with 0.1% Triton in PBS for five
minutes. The slides were overlayed with mounting media dropwise with Biomedica -Gel
Mount™ (Biomedica; Containing Anti-Quenching Agent). Cells were examined at 10x
magnification using the Nikon TE300 equipped with FITC filter (535nm).

30

Results - HGPRBMY2 constitutively activates gene expression through the NFAT response element.

There is strong evidence that certain GPCRs exhibit a cDNA concentration-
35 dependent constitutive activity through cAMP response element (CRE) luciferase
reporters (Chen et al., 1999). In an effort to demonstrate functional coupling of
HGPRBMY2 to known GPCR second messenger pathways, the HGPRBMY2

polypeptide was expressed at high constitutive levels in the Cho-NFAT/CRE cell line.

5 To this end, the HGPRBMY2 cDNA was PCR amplified and subcloned into the pcDNA3.1 hygroTM mammalian expression vector as described herein. Early passage Cho-NFAT/CRE cells were then transfected with the resulting pcDNA3.1 hygroTM / HGPRBMY2 construct. Transfected and non-transfected Cho-NFAT/CRE cells (control)
10 were loaded with the CCF2 substrate and stimulated with 10 nM PMA, and 1 uM Thapsigargin (NFAT stimulator) or 10 uM Forskolin (CRE stimulator) to fully activate the NFAT/CRE element. The cells were then analyzed for fluorescent emission by FACS.

The FACS profile demonstrates the constitutive activity of HGPRBMY2 in the Cho-NFAT/CRE line as evidenced by the significant population of cells with blue
15 fluorescent emission at 447 nm (see Figure 12: Blue Cells). As expected, the NFAT/CRE response element in the untransfected control cell line was not activated (i.e., beta lactamase not induced), enabling the CCF2 substrate to remain intact, and resulting in the green fluorescent emission at 518 nm (see Figure 11-Green Cells). A very low level of leaky Beta Lactamase expression was detectable as evidenced by the small population of
20 cells emitting at 447 nm. Analysis of a stable pool of cells transfected with HGPRBMY2 revealed constitutive coupling of the cell population to the NFAT/CRE response element, activation of Beta Lactamase and cleavage of the substrate (Figure 12-Blue Cells). These results demonstrate that overexpression of HGPRBMY2 leads to constitutive coupling
25 of signaling pathways known to be mediated by Gq/11 or Gs coupled receptors that converge to activate either the NFAT or CRE response elements respectively (Boss et al., 1996; Chen et al., 1999).

In an effort to further characterize the observed functional coupling of the HGPRBMY2 polypeptide, its ability to couple to the cAMP response element (CRE)
30 independent of the NFAT response element was examined. To this end, HEK-CRE cell line that contained only the integrated 3XCRE linked to the Beta-Lactamase reporter was transfected with the pcDNA3.1 hygroTM / HGPRBMY2 construct. Analysis of the fluorescence emission from this stable pool showed that HGPRBMY2 does not
35 constitutively couple to the cAMP mediated second messenger pathways (see Figure 13). Experiments have shown that known Gs coupled receptors do demonstrate constitutive activation when overexpressed in the HEK-CRE cell line. For example, direct activation of adenylate cyclase using 10 uM Forskolin has been shown to activate CRE and the

subsequent induction of Beta-Lactamase in the HEK-CRE cell line (data not shown). In
5 conclusion, the results are consistent with HGPRBMY2 representing a functional GPCR
analogous to known Gq coupled receptors. Therefore, constitutive expression of
HGPRBMY2 in the CHO Nfat/CRE cell line leads to NFAT activation through
accumulation of intracellular Ca^{2+} as has been demonstrated for the M3 muscarinic
10 receptor (Boss et al., 1996).

15 In preferred embodiments, the HGPRBMY2 polynucleotides and polypeptides,
including agonists, antagonists, and fragments thereof, are useful for modulating
intracellular Ca^{2+} levels, modulating Ca^{2+} sensitive signaling pathways, and modulating
NFAT element associated signaling pathways.

15 Demonstration of Cell Surface Expression:

HGPRBMY2 was tagged at the C-terminus using the Flag epitope and inserted
into the pcDNA3.1 hygroTM expression vector, as described herein.
Immunocytochemistry of Cho Nfat-CRE cell lines transfected with the Flag-tagged
20 HGPRBMY2 construct with FITC conjugated Anti Flag monoclonal antibody
demonstrated that HGPRBMY2 is indeed a cell surface receptor. The
immunocytochemistry also confirmed expression of the HGPRBMY2 in the Cho Nfat-
CRE cell lines. Briefly, Cho Nfat-CRE cell lines were transfected with pcDNA3.1 hygro
25 TM / HGPRBMY2-Flag vector, fixed with 70% methanol, and permeablized with
0.1% TritonX100. The cells were then blocked with 1% Serum and incubated with a FITC
conjugated Anti Flag monoclonal antibody at 1:50 dilution in PBS-Triton. The cells were
then washed several times with PBS-Triton, overlayed with mounting solution, and
fluorescent images were captured (see Figure 14). The control cell line, non-transfected
30 ChoNfat CRE cell line, exhibited no detectable background fluorescence (Data not
shown). The BMY2 -FLAG tagged expressing Cho Nfat CRE line exhibited specific
plasma membrane expression as indicated (Panel B). These data provide clear evidence
that BMY2 is expressed at the plasma membrane. Plasma membrane localization in
consistent with HGPRBMY2 representing a 7 transmembrane domain containing GPCR.
35 Taken together, the data indicates that HGPRBMY2 is a cell surface GPCR that functions
through increases in Ca^{2+} signal transduction pathways.

Screening Paradigm

5 The Aurora Beta-Lactamase technology provides a clear path for identifying agonists and antagonists of the HGPRBMY2 polypeptide. Cell lines that exhibit a range of constitutive coupling activity have been identified by sorting through HGPRBMY2 transfected cell lines using the FACS Vantage SE (see Figure 15). For example, cell lines
10 have been sorted that have an intermediate level of HGPRBMY2 expression, which also correlates with an intermediate coupling response, using the LJI analyst. Such cell lines will provide the opportunity to screen, indirectly, for both agonists and antagonists of HGPRBMY2 by looking for inhibitors that block the beta lactamase response, or agonists that increase the beta lactamase response. As described herein, modulating the expression
15 level of beta lactamase directly correlates with the level of cleaved CCR2 substrate. For example, this screening paradigm has been shown to work for the identification of modulators of a known GPCR, 5HT6, that couples through Adenylate Cyclase, in addition to, the identification of modulators of the 5HT2c GPCR, that couples through changes in $[Ca^{2+}]_i$. The data shown below represent cell lines that have been engineered
20 with the desired pattern of HGPRBMY2 expression to enable the identification of potent small molecule agonists and antagonists. HGPRBMY2 modulator screens may be carried out using a variety of high throughput methods known in the art, though preferably using the fully automated Aurora UHTSS system. The uninduced, HGPRBMY2 transfected
25 Cho Nfat-CRE cell line represents the relative background level of beta lactamase expression (Figure 15; panel a). Following treatment with a cocktail of 10nM Forskolin, 1uM Thapsigargin, and 100 nM PMA (Figure 15; F/T/P; panel b), the cells fully activate the CRE-NFAT response element demonstrating the dynamic range of the assay. Panel C (Figure 15) represents a HGPRBMY2 transfected Cho Nfat-CRE cell line that shows
30 an intermediate level of beta lactamase expression post F/T/P stimulation, while panel D (Figure 15) represents a HGPRBMY2 transfected Cho Nfat-CRE cell line that shows a high level of beta lactamase expression post F/T/P stimulation.

 In preferred embodiments, the HGPRBMY2 transfected Cho Nfat-CRE cell lines of the present invention are useful for the identification of agonists and antagonists of the
35 HGPRBMY2 polypeptide. Representative uses of these cell lines would be their inclusion in a method of identifying HGPRBMY2 agonists and antagonists. Preferably, the cell lines are useful in a method for identifying a compound that modulates the biological

activity of the HGPRBMY2 polypeptide, comprising the steps of (a) combining a
5 candidate modulator compound with a host cell expressing the HGPRBMY2 polypeptide
having the sequence as set forth in SEQ ID NO:14; and (b) measuring an effect of the
candidate modulator compound on the activity of the expressed HGPRBMY2
polypeptide. Representative vectors expressing the HGPRBMY2 polypeptide are
referenced herein (e.g., pcDNA3.1 hygroTM) or otherwise known in the art.

10 The cell lines are also useful in a method of screening for a compound that is
capable of modulating the biological activity of HGPRBMY2 polypeptide, comprising
the steps of: (a) determining the biological activity of the HGPRBMY2 polypeptide in
the absence of a modulator compound; (b) contacting a host cell expression the
15 HGPRBMY2 polypeptide with the modulator compound; and (c) determining the
biological activity of the HGPRBMY2 polypeptide in the presence of the modulator
compound; wherein a difference between the activity of the HGPRBMY2 polypeptide
in the presence of the modulator compound and in the absence of the modulator
compound indicates a modulating effect of the compound. Additional uses for these cell
20 lines are described herein or otherwise known in the art

- 25 1. Rees, S., Brown, S., Stables, J.: Reporter gene systems for the study of G Protein
Coupled Receptor signalling in mammalian cells. In Milligan G. (ed.): Signal
Transduction: A practical approach. Oxford: Oxford University Press, 1999: 171-221.
2. Alam, J., Cook, J.L.: Reporter Genes: Application to the study of mammalian gene
transcription. Anal. Biochem. 1990; 188: 245-254.
- 30 3. Selbie, L.A. and Hill, S.J.: G protein-coupled receptor cross-talk: The fine-tuning of
multiple receptor-signaling pathways. TIPS. 1998; 19: 87-93.
4. Boss, V., Talpade, D.J., and Murphy, T.J.: Induction of NFAT mediated transcription
by Gq-coupled Receptors in lymphoid and non-lymphoid cells. JBC. 1996; 271:
35 10429-10432.
5. George, S.E., Bungay, B.J., and Naylor, L.H.: Functional coupling of endogenous

- serotonin (5-HT_{1B}) and calcitonin (C_{1a}) receptors in Cho cells to a cyclic AMP-
5 responsive luciferase reporter gene. *J. Neurochem.* 1997; 69: 1278-1285.
6. Suto, CM, Igna DM: Selection of an optimal reporter for cell-based high throughput
screening assays. *J. Biomol. Screening.* 1997; 2: 7-12.
- 10 7. Zlokarnik, G., Negulescu, P.A., Knapp, T.E., More, L., Burres, N., Feng, L., Whitney,
M., Roemer, K., and Tsien, R. Y. Quantitation of transcription and clonal selection
of single living cells with a B-Lactamase Reporter. *Science.* 1998; 279: 84-88.
- 15 8. S. Fiering et. al., *Genes Dev.* 4, 1823 (1990).
9. J. Karttunen and N. Shastri, *PNAS* 88, 3972 (1991).
10. Hawes, B. E., Luttrell. L.M., van Biesen, T., and Lefkowitz, R.J. (1996) *JBC* 271,
20 12133-12136.
11. Gilman, A.G. (1987) *Annul. Rev. Biochem.* 56, 615-649.
- 25 12. Maniatis et al.,
13. Salcedo, R., Ponce, M.L., Young, H.A., Wasserman, K., Ward, J.M., Kleinman, H.K.,
Oppenheim, J.J., Murphy, W.J. Human endothelial cells express CCR2 and respond
to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. *Blood.* 2000;
30 96 (1): 34-40.
14. Sica, A., Saccani, A., Bottazzi, B., Bernasconi, S., Allavena, P., Gaetano, B.,
LaRossa, G., Scotton, C., Balkwill F., Mantovani, A. Defective expression of the
monocyte chemotactic protein 1 receptor CCR2 in macrophages associated with
35 human ovarian carcinoma. *J. Immunology.* 2000; 164: 733-8.
15. Kypson, A., Hendrickson, S., Akhter, S., Wilson, K., McDonald, P., Lilly, R., Dolber,

- 5 P., Glower, D., Lefkowitz, R., Koch, W. Adenovirus-mediated gene transfer of the B2 AR to donor hearts enhances cardiac function. *Gene Therapy*. 1999; 6: 1298-304.
16. Dorn, G.W., Tepe, N.M., Lorenz, J.N., Kock, W.J., Liggett, S.B. Low and high level transgenic expression of B2AR differentially affect cardiac hypertrophy and function in Galpha q-overexpressing mice. *PNAS*. 1999; 96: 6400-5.
- 10 17. J. Wess. G protein coupled receptor: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition.
- 15 18. Whitney, M, Rockenstein, E, Cantin, G., Knapp, T., Zlokarnik, G., Sanders, P., Durick, K., Craig, F.F., and Negulescu, P.A. A genome-wide functional assay of signal transduction in living mammalian cells. 1998. *Nature Biotech.* 16: 1329-1333.
19. BD Biosciences: FACS Vantage SE Training Manual. Part Number 11-11020-00
20 Rev. A. August 1999.
20. Chen, G., Jaywickreme, C., Way, J., Armour S., Queen K., Watson., C., Ignar, D., Chen, W.J., Kenakin, T. Constitutive Receptor systems for drug discovery. *J. Pharmacol. Toxicol. Methods* 1999; 42: 199-206.
- 25

30 **Example 10 - Phage Display Methods for Identifying Peptide Ligands or Modulators of Orphan GPCRs**

Creation of Peptide Libraries.

Two types of libraries may be created: i.) libraries of 12- and 15 mer peptides for finding peptides that may function as (ant-)agonists and ii.) libraries of peptides with 23-
35 33 random residues that are for finding natural ligands through database searches.

The 15 mer library may be i.) an aliquot of the fUSE5-based 15 mer library originally constructed by GP Smith (Scott, JK and Smith, GP. 1990, *Science* 249, 386-

390). Such a library may be made essentially as described therein, or ii.) a library that is
5 constructed at Bristol-Myers Squibb in vector M13KE (New England Biolabs) using a
single-stranded library oligonucleotide extension method (S.S. Sidhu, H.B. Lowman,
B.C. Cunningham, J.A. Wells: Methods Enzymol., 2000, vol 328, 333-363).

The 12 mer library is an aliquot of the M13KE-based 'PhD' 12 mer library (New
10 England Biolabs).

The libraries with 27-33 random residues are also constructed at Bristol-Myers
Squibb in vector M13KE (New England Biolabs) using the method described in (S.S.
Sidhu, H.B. Lowman, B.C. Cunningham, J.A. Wells: Methods Enzymol., 2000, vol 328,
333-363).

15 All libraries in vector M13KE utilize the standard NNK motif to encode the
specified number of random residues, where $N = A+G+C+T$ and where $K=G+T$.

Sequencing of bound phage:

Standard procedure. Phage in eluates are infected into E. coli host strain (TG1 for
20 fUSE5-based 15 mer library; ER2738 (New England Biolabs) for all M13KE-based
libraries) and are plated for single colonies (fUSE5 vector) or plaques (all M13KE-based
libraries). Colonies are grown in liquid and sequenced by standard procedure which
involves 1.) generating PCR product with suitable primers that anneal adjacent to the
25 library segments in the vectors and 2.) sequencing of the PCR products using one primer
of each PCR primer pair. Sequences are analyzed for homologies by visual inspection or
by using the Vector NTI alignment tool.

Peptide Synthesis

30 Peptides are synthesized on Fmoc-Knorr amide resin [N-(9-
fluorenyl)methoxycarbonyl-Knorr amide-resin, Midwest Biotech, Fishers, Indiana] with
an Applied Biosystems (Foster City, California) model 433A synthesizer and the *FastMoc*
chemistry protocol (0.25mmol scale) supplied with the instrument. Amino acids are
double coupled as their N-alpha-Fmoc- derivatives and reactive side chains are protected
35 as follows: Asp, Glu: t-Butyl ester (OtBu); Ser, Thr, Tyr: t-Butyl ether (tBu); Asn, Cys,
Gln, His: Triphenylmethyl (Trt); Lys, Trp: t-Butyloxycarbonyl (Boc); Arg: 2,2,4,6,7-
Pentamethyldihydrobenzofuran-5-sulfonyl (Pbf). After the final double coupling cycle,

the N-terminal Fmoc group is removed by the multi-step treatment with piperidine in N-Methylpyrrolidone described by the manufacturer. The N-terminal free amines are then
5 treated with 10% acetic anhydride, 5% Diisopropylamine in N-Methylpyrrolidone to yield the N-acetyl-derivative. The protected peptidyl-resins are simultaneously deprotected and removed from the resin by standard methods. The lyophilized peptides are purified on C₁₈
10 to apparent homogeneity as judged by RP-HPLC analysis. Predicted peptide molecular weights are verified by electrospray mass spectrometry.
(J. Biol. Chem.. vol. 273, pp.12041-12046, 1998)

Cyclic analogs are prepared from the crude linear products. The cystine disulfide
15 may be formed using one of the following methods:

Method 1: A sample of the crude peptide is dissolved in water at a concentration of 0.5 mg/mL and the pH adjusted to 8.5 with NH₄OH. The reaction is stirred, open to room air, and monitored by RP-HPLC.

20 Once complete, the reaction is brought to pH 4 with acetic acid and lyophilized. The product is purified and characterized as above.

Method 2: A sample of the crude peptide is dissolved at a concentration of 0.5mg/mL in
25 5% acetic acid. The pH is adjusted to 6.0 with NH₄OH. DMSO (20% by volume) is added and the reaction is stirred overnight. After analytical RP-HPLC analysis, the reaction is diluted with H₂O and triple lyophilized to remove DMSO. The crude product is purified by preparative RP-HPLC. (JACS. vol. 113, 6657, 1991)

30 *HGPRBMY2 Peptide Modulators of The Present Invention.*

GDFWYEACESSCAFW (SEQ ID NO:32)

35 LEWGSDVFYDVYDCC (SEQ ID NO:33)

CLRSGTGCAFQLYRF (SEQ ID NO:34)

FAGQIIWYDALDTLM (SEQ ID NO:35)

5

Assessing Affect of Peptides on GPCR Function.

The effect of any one of these peptides on the function of the GPCR of the present invention may be determined by adding an effective amount of each peptide to each functional assay. Representative functional assays are described more specifically herein.

10

Uses of the peptide modulators of the present invention.

The aforementioned peptides of the present invention are useful for a variety of purposes, though most notably for modulating the function of the GPCR of the present invention, and potentially with other GPCRs of the same G-protein coupled receptor subclass (e.g., peptide receptors, adrenergic receptors, purinergic receptors, etc.), and/or other subclasses known in the art. For example, the peptide modulators of the present invention may be useful as HGPRBMY2 agonists. Alternatively, the peptide modulators of the present invention may be useful as HGPRBMY2 antagonists of the present invention. In addition, the peptide modulators of the present invention may be useful as competitive inhibitors of the HGPRBMY2 cognate ligand(s), or may be useful as non-competitive inhibitors of the HGPRBMY2 cognate ligand(s).

20

Furthermore, the peptide modulators of the present invention may be useful in assays designed to either deorphan the HGPRBMY2 polypeptide of the present invention, or to identify other agonists or antagonists of the HGPRBMY2 polypeptide of the present invention, particularly small molecule modulators.

25

Example 11 – Alternative Method Of Assessing The Ability Of HGPRBMY1 or HGPRBMY2 To Serve As A GPCR Receptor.

30

The activity of the HGPRBMY1 or HGPRBMY2 polypeptides may be measured using an assay based upon the property of some known GPCRs to support proliferation in vitro of fibroblasts and tumor cells under serum-free conditions (Chiquet Ehrismann, R. et al. (1986) Cell 47: 131-139). Briefly, wells in 96 well cluster plates (Falcon, Fisher Scientific, Santa Clara CA) are coated with HGPRBMY1 or HGPRBMY2 polypeptides by incubation with solutions at 50-100 Rg/ml for 15 min at ambient temperature. The coating solution is aspirated, and the wells washed with Dulbecco's medium before cells

35

are plated. Rat fibroblast cultures or rat mammary tumor cells are prepared as described
5 and plated at a density of 104-105 cells/ml in Dulbecco's medium supplemented with
10% fetal calf serum (FCS).

After three days the media are removed, and the cells washed three times with
phosphatebuffered saline (PBS) before the addition of serum-free Dulbecco's medium
containing 0.25 mg/ml bovine serum albumin (BSA, Fraction V, Sigma Chemical, St.
10 Louis, MO). After 2 days the medium is aspirated, and 100 μ l of [3H] thymidine (NEN)
at 2 μ Ci/ml in fresh Dulbecco's medium containing 0.25 mg/ml BSA added. Parallel
plates are fixed and stained to determine cell numbers. After 16 hr, the medium is
aspirated, the cell layer washed with PBS, and the 10% trichloroacetic acid-precipitable
15 counts in the cell layer determined by liquid scintillation counting of radioisotope
(normalized to relative cell numbers; Chiquet-Ehrismann, R. et al. (1986) supra). The
rates of cell proliferation and [3H] thymidine uptake are proportional to the levels of
GCRP in the sample.

Alternatively, the assay for HGPRBMY1 or HGPRBMY2 polypeptide activity
20 is based upon the property of CD97/Emrl GPCR family proteins to modulate G protein-
activated second messenger signal transduction pathways (e. g., cAMP; Gaudin, P. et al.
(1998) J. Biol. Chem., 273: 4990-4996). A plasmid encoding the full length HGPRBMY1
or HGPRBMY2 polypeptide is transfected into a mammalian cell line (e. g., COS-7 or
25 Chinese hamster ovary (CHO-K1) cell lines) using methods well-known in the art.
Transfected cells are grown in 12-well trays in culture medium containing 2% FCS for
48 hours, the culture medium is discarded, then the attached cells are gently washed with
PBS. The cells are then incubated in culture medium with 10% FCS or 2% FCS for 30
minutes, then the medium is removed and cells lysed by treatment with 1 M perchloric
30 acid. The cAMP levels in the lysate are measured by radioimmunoassay using methods
well-known in the art. Changes in the levels of cAMP in the lysate from 10% FCS-treated
cells compared with those in 2% FCS-treated cells are proportional to the amount of the
HGPRBMY1 or HGPRBMY2 polypeptide present in the transfected cells.

35 **Example 12 – Method Of Assessing The Physiological Function Of The
HGPRBMY1 or HGPRBMY2 Polypeptide At The Cellular Level.**

The physiological function of the HGPRBMY1 or HGPRBMY2 polypeptide may

be assessed by expressing the sequences encoding HGPRBMY1 or HGPRBMY2 at
5 physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned
into a mammalian expression vector containing a strong promoter that drives high levels
of cDNA expression (examples are provided elsewhere herein). Vectors of choice include
pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of
10 which contain the cytomegalovirus promoter. 5-10, ug of recombinant vector are
transiently transfected into a human cell line, preferably of endothelial or hematopoietic
origin, using either liposome formulations or electroporation. 1-2ug of an additional
plasmid containing sequences encoding a marker protein are cotransfected. Expression
of a marker protein provides a means to distinguish transfected cells from nontransfected
15 cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker
proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a
CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based
technique, is used to identify transfected cells expressing GFP or CD64-GFP and to
evaluate the apoptotic state of the cells and other cellular properties. FCM detects and
20 quantifies the uptake of fluorescent molecules that diagnose events preceding or
coincident with cell death. These events include changes in nuclear DNA content as
measured by staining of DNA with propidium iodide; changes in cell size and granularity
as measured by forward light scatter and 90 degree side light scatter; down-regulation of
DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in
25 expression of cell surface and intracellular proteins as measured by reactivity with
specific antibodies; and alterations in plasma membrane composition as measured by the
binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow
cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York
30 NY.

The influence of HGPRBMY1 or HGPRBMY2 polypeptides on gene expression
can be assessed using highly purified populations of cells transfected with sequences
encoding HGPRBMY1 or HGPRBMY2 and either CD64 or CD64-GFP. CD64 and
CD64-GFP are expressed on the surface of transfected cells and bind to conserved
35 regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated
from nontransfected cells using magnetic beads coated with either human IgG or antibody
against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using

methods well known by those of skill in the art. Expression of mRNA encoding
5 HGPRBMY1 or HGPRBMY2 polypeptides and other genes of interest can be analyzed
by northern analysis or microarray techniques.

**Example 13 – Method Of Assessing The Physiological Function Of The
HGPRBMY1 or HGPRBMY2 Polypeptides In Xenopus Oocytes.**

10 Capped RNA transcripts from linearized plasmid templates encoding the receptor
cDNAs of the invention are synthesized in vitro with RNA polymerases in accordance
with standard procedures.

In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml.
15 Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are
obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a
microinjection apparatus. Two electrode voltage clamps are used to measure the currents
from individual Xenopus oocytes in response to agonist exposure. Recordings are made
in Ca²⁺ free Barth's medium at room temperature.

20 In a preferred embodiment, such a system can be used to screen known ligands
and tissue/cell extracts for activating ligands. A number of GPCR ligands are known in
the art and are encompassed by the present invention (see, for example, The G-Protein
Linked Receptor Facts Book, referenced elsewhere herein).

25 **Example 14 - Method Of Assessing The Physiological Function Of The
HGPRBMY1 or HGPRBMY2 Polypeptides Using Microphysiometric Assays.**

Activation of a wide variety of secondary messenger systems results in extrusion
of small amounts of acid from a cell. The acid formed is largely as a result of the
30 increased metabolic activity required to fuel the intracellular signaling process. The pH
changes in the media surrounding the cell are very small but are detectable by the
CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The
CYTOSENSOR is thus capable of detecting the activation of a receptor that is coupled
35 to an energy utilizing intracellular signaling pathway such as the G-protein coupled
receptor of the present invention.

Example 15 - Method Of Assessing The Physiological Function Of The

HGPRBMY1 or HGPRBMY2 Polypeptides Using Calcium And Camp Functional**5 Assays.**

A well known observation in the art relates to the fact that GPCR receptors which are expressed in HEK 293 cells have been shown to be functionally couple – leading to subsequent activation of phospholipase C (PLC) and calcium mobilization, and/or cAMP stimulation or inhibition.

10 Based upon the above, calcium and cAMP assays may be useful in assessing the ability of HGPRBMY1 or HGPRBMY2 to serve as a GPCR. Briefly, basal calcium levels in the HEK 293 cells in HGPRBMY1 or HGPRBMY2-transfected or vector control cells can be observed to determine whether the levels fall within a normal physiological range, 15 100 nM to 200 nM. HEK 293 cells expressing recombinant receptors are then loaded with fura 2 and in a single day selected GPCR ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant HGPRBMY1 or HGPRBMY2 receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists 20 presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing the HGPRBMY1 or HGPRBMY2 receptor.

25 Example 16 – Method Of Screening For Compounds That Interact With The HGPRBMY1 or HGPRBMY2 Polypeptide.

The following assays are designed to identify compounds that bind to the HGPRBMY1 or HGPRBMY2 polypeptide, bind to other cellular proteins that interact with the HGPRBMY1 or HGPRBMY2 polypeptide, and to compounds that interfere with 30 the interaction of the HGPRBMY1 or HGPRBMY2 polypeptide with other cellular proteins.

Such compounds can include, but are not limited to, other cellular proteins. Specifically, such compounds can include, but are not limited to, peptides, such as, for example, soluble peptides, including, but not limited to Ig-tailed fusion peptides, 35 comprising extracellular portions of HGPRBMY1 or HGPRBMY2 polypeptide transmembrane receptors, and members of random peptide libraries (see, e.g., Lam, K. S. et al., 1991, Nature 354:82-84; Houghton, R. et al., 1991, Nature 354:84-86), made of

D-and/or L-configuration amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate phosphopeptide libraries; see, e.g., Songyang, Z., et al., 1993, Cell 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')₂ and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

Compounds identified via assays such as those described herein can be useful, for example, in elaborating the biological function of the HGPRBMY1 or HGPRBMY2 polypeptide, and for ameliorating symptoms of tumor progression, for example. In instances, for example, whereby a tumor progression state or disorder results from a lower overall level of HGPRBMY1 or HGPRBMY2 expression, HGPRBMY1 or HGPRBMY2 polypeptide, and/or HGPRBMY1 or HGPRBMY2 polypeptide activity in a cell involved in the tumor progression state or disorder, compounds that interact with the HGPRBMY1 or HGPRBMY2 polypeptide can include ones which accentuate or amplify the activity of the bound HGPRBMY1 or HGPRBMY2 polypeptide. Such compounds would bring about an effective increase in the level of HGPRBMY1 or HGPRBMY2 polypeptide activity, thus ameliorating symptoms of the tumor progression disorder or state. In instances whereby mutations within the HGPRBMY1 or HGPRBMY2 polypeptide cause aberrant HGPRBMY1 or HGPRBMY2 polypeptides to be made which have a deleterious effect that leads to tumor progression, compounds that bind HGPRBMY1 or HGPRBMY2 polypeptide can be identified that inhibit the activity of the bound HGPRBMY1 or HGPRBMY2 polypeptide. Assays for testing the effectiveness of such compounds are known in the art and discussed, elsewhere herein.

Example 17 – Method Of Screening, In Vitro, Compounds That Bind To The HGPRBMY1 or HGPRBMY2 Polypeptide.

In vitro systems can be designed to identify compounds capable of binding the HGPRBMY1 or HGPRBMY2 polypeptide of the invention. Compounds identified can be useful, for example, in modulating the activity of wild type and/or mutant HGPRBMY1 or HGPRBMY2 polypeptide, preferably mutant HGPRBMY1 or HGPRBMY2 polypeptide, can be useful in elaborating the biological function of the HGPRBMY1 or HGPRBMY2 polypeptide, can be utilized in screens for identifying

compounds that disrupt normal HGPRBMY1 or HGPRBMY2 polypeptide interactions,
5 or can in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to the
HGPRBMY1 or HGPRBMY2 polypeptide involves preparing a reaction mixture of the
HGPRBMY1 or HGPRBMY2 polypeptide and the test compound under conditions and
10 for a time sufficient to allow the two components to interact and bind, thus forming a
complex which can be removed and/or detected in the reaction mixture. These assays can
be conducted in a variety of ways. For example, one method to conduct such an assay
would involve anchoring HGPRBMY1 or HGPRBMY2 polypeptide or the test substance
onto a solid phase and detecting HGPRBMY1 or HGPRBMY2 polypeptide /test
15 compound complexes anchored on the solid phase at the end of the reaction. In one
embodiment of such a method, the HGPRBMY1 or HGPRBMY2 polypeptide can be
anchored onto a solid surface, and the test compound, which is not anchored, can be
labeled, either directly or indirectly.

In practice, microtitre plates can conveniently be utilized as the solid phase. The
20 anchored component can be immobilized by non-covalent or covalent attachments. Non-
covalent attachment can be accomplished by simply coating the solid surface with a
solution of the protein and drying. Alternatively, an immobilized antibody, preferably a
monoclonal antibody, specific for the protein to be immobilized can be used to anchor
25 the protein to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the nonimmobilized component is added to the
coated surface containing the anchored component. After the reaction is complete,
unreacted components are removed (e.g., by washing) under conditions such that any
complexes formed will remain immobilized on the solid surface. The detection of
30 complexes anchored on the solid surface can be accomplished in a number of ways.
Where the previously immobilized component is pre-labeled, the detection of label
immobilized on the surface indicates that complexes were formed. Where the previously
nonimmobilized component is not pre-labeled, an indirect label can be used to detect
complexes anchored on the surface; e.g., using a labeled antibody specific for the
35 immobilized component (the antibody, in turn, can be directly labeled or indirectly
labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products

separated from unreacted components, and complexes detected; e.g., using an
5 immobilized antibody specific for HGPRBMY1 or HGPRBMY2 polypeptide or the test
compound to anchor any complexes formed in solution, and a labeled antibody specific
for the other component of the possible complex to detect anchored complexes.

10 **Example 18 – Method For Identifying A Putative Ligand For The HGCRBMY1 or
HGPRBMY2 Polypeptide.**

Ligand binding assays provide a direct method for ascertaining receptor
pharmacology and are adaptable to a high throughput format. A panel of known GPCR
purified ligands may be radiolabeled to high specific activity (50-2000 Ci/mmol) for
15 binding studies. A determination is then made that the process of radiolabeling does not
diminish the activity of the ligand towards its receptor. Assay conditions for buffers, ions,
pH and other modulators such as nucleotides are optimized to establish a workable signal
to noise ratio for both membrane and whole cell receptor sources. For these assays,
specific receptor binding is defined as total associated radioactivity minus the
20 radioactivity measured in the presence of an excess of unlabeled competing ligand.
Where possible, more than one competing ligand is used to define residual nonspecific
binding.

A number of GPCR ligands are known in the art and are encompassed by the
25 present invention (see, for example, The G-Protein Linked Receptor Facts Book,
referenced elsewhere herein).

Alternatively, the HGPRBMY1 or HGPRBMY2 polypeptide of the present
invention may also be functionally screened (using calcium, cAMP, microphysiometer,
oocyte electrophysiology, etc., functional screens) against tissue extracts to identify
30 natural ligands. Extracts that produce positive functional responses can be sequentially
subfractionated until an activating ligand is isolated identified using methods well known
in the art, some of which are described herein.

35 **Example 19 – Method Of Identifying Compounds That Interfere With HGPRBMY1
or HGPRBMY2 Polypeptide/Cellular Product Interaction.**

The HGPRBMY1 or HGPRBMY2 polypeptide of the invention can, in vivo,

interact with one or more cellular or extracellular macromolecules, such as proteins. Such
5 macromolecules include, but are not limited to, polypeptides, particularly GPCR ligands,
and those products identified via screening methods described, elsewhere herein. For the
purposes of this discussion, such cellular and extracellular macromolecules are referred
to herein as "binding partner(s)". For the purpose of the present invention, "binding
10 partner" may also encompass polypeptides, small molecule compounds, polysaccharides,
lipids, and any other molecule or molecule type referenced herein. Compounds that
disrupt such interactions can be useful in regulating the activity of the HGPRBMY1 or
HGPRBMY2 polypeptide, especially mutant HGPRBMY1 or HGPRBMY2 polypeptide.
Such compounds can include, but are not limited to molecules such as antibodies,
15 peptides, and the like described in elsewhere herein.

The basic principle of the assay systems used to identify compounds that interfere
with the interaction between the HGPRBMY1 or HGPRBMY2 polypeptide and its
cellular or extracellular binding partner or partners involves preparing a reaction mixture
containing the HGPRBMY1 or HGPRBMY2 polypeptide, and the binding partner under
20 conditions and for a time sufficient to allow the two products to interact and bind, thus
forming a complex. In order to test a compound for inhibitory activity, the reaction
mixture is prepared in the presence and absence of the test compound. The test compound
can be initially included in the reaction mixture, or can be added at a time subsequent to
the addition of HGPRBMY1 or HGPRBMY2 polypeptide and its cellular or extracellular
25 binding partner. Control reaction mixtures are incubated without the test compound or
with a placebo. The formation of any complexes between the HGPRBMY1 or
HGPRBMY2 polypeptide and the cellular or extracellular binding partner is then
detected. The formation of a complex in the control reaction, but not in the reaction
30 mixture containing the test compound, indicates that the compound interferes with the
interaction of the HGPRBMY1 or HGPRBMY2 polypeptide and the interactive binding
partner. Additionally, complex formation within reaction mixtures containing the test
compound and normal HGPRBMY1 or HGPRBMY2 polypeptide can also be compared
to complex formation within reaction mixtures containing the test compound and mutant
35 HGPRBMY1 or HGPRBMY2 polypeptide. This comparison can be important in those
cases wherein it is desirable to identify compounds that disrupt interactions of mutant but
not normal HGPRBMY1 or HGPRBMY2 polypeptide.

The assay for compounds that interfere with the interaction of the HGPRBMY1
5 or HGPRBMY2 polypeptide and binding partners can be conducted in a heterogeneous
or homogeneous format. Heterogeneous assays involve anchoring either the HGPRBMY1
or HGPRBMY2 polypeptide or the binding partner onto a solid phase and detecting
complexes anchored on the solid phase at the end of the reaction. In homogeneous assays,
10 the entire reaction is carried out in a liquid phase. In either approach, the order of addition
of reactants can be varied to obtain different information about the compounds being
tested. For example, test compounds that interfere with the interaction between the
HGPRBMY1 or HGPRBMY2 polypeptide and the binding partners, e.g., by competition,
can be identified by conducting the reaction in the presence of the test substance; i.e., by
15 adding the test substance to the reaction mixture prior to or simultaneously with the
HGPRBMY1 or HGPRBMY2 polypeptide and interactive cellular or extracellular
binding partner. Alternatively, test compounds that disrupt preformed complexes, e.g.
compounds with higher binding constants that displace one of the components from the
complex, can be tested by adding the test compound to the reaction mixture after
20 complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the HGPRBMY1 or HGPRBMY2
polypeptide or the interactive cellular or extracellular binding partner, is anchored onto
a solid surface, while the non-anchored species is labeled, either directly or indirectly. In
25 practice, microtitre plates are conveniently utilized. The anchored species can be
immobilized by non-covalent or covalent attachments. Non-covalent attachment can be
accomplished simply by coating the solid surface with a solution of the HGPRBMY1 or
HGPRBMY2 polypeptide or binding partner and drying. Alternatively, an immobilized
antibody specific for the species to be anchored can be used to anchor the species to the
30 solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed
to the coated surface with or without the test compound. After the reaction is complete,
unreacted components are removed (e.g., by washing) and any complexes formed will
remain immobilized on the solid surface. The detection of complexes anchored on the
35 solid surface can be accomplished in a number of ways. Where the non-immobilized
species is pre-labeled, the detection of label immobilized on the surface indicates that
complexes were formed. Where the non-immobilized species is not pre-labeled, an

indirect label can be used to detect complexes anchored on the surface; e.g., using a
5 labeled antibody specific for the initially non-immobilized species (the antibody, in turn,
can be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending
upon the order of addition of reaction components, test compounds which inhibit
complex formation or which disrupt preformed complexes can be detected.

10 Alternatively, the reaction can be conducted in a liquid phase in the presence or
absence of the test compound, the reaction products separated from unreacted
components, and complexes detected; e.g., using an immobilized antibody specific for
one of the binding components to anchor any complexes formed in solution, and a labeled
antibody specific for the other partner to detect anchored complexes. Again, depending
15 upon the order of addition of reactants to the liquid phase, test compounds which inhibit
complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used.
In this approach, a preformed complex of the HGPRBMY1 or HGPRBMY2 polypeptide
and the interactive cellular or extracellular binding partner product is prepared in which
20 either the HGPRBMY1 or HGPRBMY2 polypeptide or their binding partners are labeled,
but the signal generated by the label is quenched due to complex formation (see, e.g.,
U.S. Pat. No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays).
The addition of a test

25 substance that competes with and displaces one of the species from the preformed
complex will result in the generation of a signal above background. In this way, test
substances which disrupt HGPRBMY1 or HGPRBMY2 polypeptide -cellular or
extracellular binding partner interaction can be identified.

In a particular embodiment, the HGPRBMY1 or HGPRBMY2 polypeptide can
30 be prepared for immobilization using recombinant DNA techniques known in the art. For
example, the HGPRBMY1 or HGPRBMY2 polypeptide coding region can be fused to
a glutathione-S-transferase (GST) gene using a fusion vector such as pGEX-5X-1, in such
a manner that its binding activity is maintained in the resulting fusion product. The
interactive cellular or extracellular product can be purified and used to raise a monoclonal
35 antibody, using methods routinely practiced in the art and described above. This antibody
can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely
practiced in the art. In a heterogeneous assay, e.g., the GST- HGPRBMY1 or

HGPRBMY2 polypeptide fusion product can be anchored to glutathione-agarose beads.

5 The interactive cellular or extracellular binding partner product can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system and allowed to bind to the complexed components. The interaction between the HGPRBMY1 or HGPRBMY2
10 polypeptide and the interactive cellular or extracellular binding partner can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. A successful inhibition of the interaction by the test compound will result in a decrease in measured radioactivity.

15 Alternatively, the GST- HGPRBMY1 or HGPRBMY2 polypeptide fusion product and the interactive cellular or extracellular binding partner product can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added either during or after the binding partners are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed
20 away. Again the extent of inhibition of the binding partner interaction can be detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of the HGPRBMY1 or
25 HGPRBMY2 polypeptide product and the interactive cellular or extracellular binding partner (in case where the binding partner is a product), in place of one or both of the full length products.

Any number of methods routinely practiced in the art can be used to identify and isolate the protein's binding site. These methods include, but are not limited to,
30 mutagenesis of one of the genes encoding one of the products and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the gene encoding the second species in the complex can be selected. Sequence analysis of the genes encoding the respective products will reveal the mutations that correspond to the region of the product involved in interactive binding. Alternatively, one product can
35 be anchored to a solid surface using methods described in this Section above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide

comprising the binding domain can remain associated with the solid material, which can
5 be isolated and identified by amino acid sequencing. Also, once the gene coding for the
cellular or extracellular binding partner product is obtained, short gene segments can be
engineered to express peptide fragments of the product, which can then be tested for
binding activity and purified or synthesized.

10 **Example 20 - Isolation Of A Specific Clone From The Deposited Sample.**

The deposited material in the sample assigned the ATCC Deposit Number cited
herein for any given cDNA clone also may contain one or more additional plasmids, each
comprising a cDNA clone different from that given clone. Thus, deposits sharing the
15 same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified
herein. Typically, each ATCC deposit sample cited herein comprises a mixture of
approximately equal amounts (by weight) of about 1-10 plasmid DNAs, each containing
a different cDNA clone and/or partial cDNA clone; but such a deposit sample may
include plasmids for more or less than 2 cDNA clones.

20 Two approaches can be used to isolate a particular clone from the deposited
sample of plasmid DNA(s) of the present invention. First, a plasmid is directly isolated
by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:1 or
SEQ ID NO:13.

25 Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using
an Applied Biosystems DNA synthesizer according to the sequence reported. The
oligonucleotide is labeled, for instance, with ³²P-(-ATP using T4 polynucleotide kinase
and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A
Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid
30 mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue
(Stratagene)) using techniques known to those of skill in the art, such as those provided
by the vector supplier or in related publications or patents cited above. The transformants
are plated on 1.5% agar plates (containing the appropriate selection agent, e.g.,
ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are
35 screened using Nylon membranes according to routine methods for bacterial colony
screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit.,

(1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques
5 known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the
SEQ ID NO:1 or SEQ ID NO:13 (i.e., within the region of SEQ ID NO:1 or SEQ ID
NO:13 bounded by the 5' NT and the 3' NT of the clone defined herein) are synthesized
and used to amplify the desired cDNA using the deposited cDNA plasmid as a template.
10 The polymerase chain reaction is carried out under routine conditions, for instance, in 25
ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction
mixture is 1.5-5 mM MgCl₂, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP,
dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of
15 PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min;
elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated
thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the
DNA band with expected molecular weight is excised and purified. The PCR product is
verified to be the selected sequence by subcloning and sequencing the DNA product.

20 The polynucleotide(s) of the present invention, the polynucleotide encoding the
polypeptide of the present invention, or the polypeptide encoded by the deposited clone
may represent partial, or incomplete versions of the complete coding region (i.e., full-
length gene). Several methods are known in the art for the identification of the 5' or 3'
25 non-coding and/or coding portions of a gene which may not be present in the deposited
clone. The methods that follow are exemplary and should not be construed as limiting the
scope of the invention. These methods include but are not limited to, filter probing, clone
enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE"
protocols that are well known in the art. For instance, a method similar to 5' RACE is
30 available for generating the missing 5' end of a desired full-length transcript. (Fromont-
Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993)).

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population
of RNA presumably containing full-length gene RNA transcripts. A primer set containing
a primer specific to the ligated RNA oligonucleotide and a primer specific to a known
35 sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-
length gene. This amplified product may then be sequenced and used to generate the full-
length gene.

This above method starts with total RNA isolated from the desired source,
5 although poly-A+ RNA can be used. The RNA preparation can then be treated with
phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA
that may interfere with the later RNA ligase step. The phosphatase should then be
inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove
10 the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5'
phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an
RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA
synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used
15 as a template for PCR amplification of the desired 5' end using a primer specific to the
ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of
interest. The resultant product is then sequenced and analyzed to confirm that the 5' end
sequence belongs to the desired gene. Moreover, it may be advantageous to optimize the
RACE protocol to increase the probability of isolating additional 5' or 3' coding or non-
20 coding sequences. Various methods of optimizing a RACE protocol are known in the art,
though a detailed description summarizing these methods can be found in B.C. Schaefer,
Anal. Biochem., 227:255-273, (1995).

An alternative method for carrying out 5' or 3' RACE for the identification of
25 coding or non-coding sequences is provided by Frohman, M.A., et al.,
Proc.Nat'l.Acad.Sci.USA, 85:8998-9002 (1988). Briefly, a cDNA clone missing either
the 5' or 3' end can be reconstructed to include the absent base pairs extending to the
translational start or stop codon, respectively. In some cases, cDNAs are missing the start
of translation, therefor. The following briefly describes a modification of this original 5'
30 RACE procedure. Poly A+ or total RNAs reverse transcribed with Superscript II
(Gibco/BRL) and an antisense or I complementary primer specific to the cDNA sequence.
The primer is removed from the reaction with a Microcon Concentrator (Amicon). The
first-strand cDNA is then tailed with dATP and terminal deoxynucleotide transferase
(Gibco/BRL). Thus, an anchor sequence is produced which is needed for PCR
35 amplification. The second strand is synthesized from the dA-tail in PCR buffer, Taq
DNA polymerase (Perkin-Elmer Cetus), an oligo-dT primer containing three adjacent
restriction sites (XhoI, SalI and ClaI) at the 5' end and a primer containing just these

restriction sites. This double-stranded cDNA is PCR amplified for 40 cycles with the
5 same primers as well as a nested cDNA-specific antisense primer. The PCR products are
size-separated on an ethidium bromide-agarose gel and the region of gel containing
cDNA products the predicted size of missing protein-coding DNA is removed. cDNA is
purified from the agarose with the Magic PCR Prep kit (Promega), restriction digested
10 with XhoI or SalI, and ligated to a plasmid such as pBluescript SKII (Stratagene) at XhoI
and EcoRV sites. This DNA is transformed into bacteria and the plasmid clones
sequenced to identify the correct protein-coding inserts. Correct 5' ends are confirmed by
comparing this sequence with the putatively identified homologue and overlap with the
partial cDNA clone. Similar methods known in the art and/or commercial kits are used
15 to amplify and recover 3' ends.

Several quality-controlled kits are commercially available for purchase. Similar
reagents and methods to those above are supplied in kit form from Gibco/BRL for both
5' and 3' RACE for recovery of full length genes. A second kit is available from Clontech
which is a modification of a related technique, SLIC (single-stranded ligation to single-
20 stranded cDNA), developed by Dumas et al., Nucleic Acids Res., 19:5227-32(1991). The
major differences in procedure are that the RNA is alkaline hydrolyzed after reverse
transcription and RNA ligase is used to join a restriction site-containing anchor primer
to the first-strand cDNA. This obviates the necessity for the dA-tailing reaction which
25 results in a polyT stretch that is difficult to sequence past.

An alternative to generating 5' or 3' cDNA from RNA is to use cDNA library
double-stranded DNA. An asymmetric PCR-amplified antisense cDNA strand is
synthesized with an antisense cDNA-specific primer and a plasmid-anchored primer.
These primers are removed and a symmetric PCR reaction is performed with a nested
30 cDNA-specific antisense primer and the plasmid-anchored primer.

RNA Ligase Protocol For Generating The 5' or 3' End Sequences To Obtain Full Length Genes

35 Once a gene of interest is identified, several methods are available for the
identification of the 5' or 3' portions of the gene which may not be present in the original
cDNA plasmid. These methods include, but are not limited to, filter probing, clone
enrichment using specific probes and protocols similar and identical to 5' and 3' RACE.

While the full-length gene may be present in the library and can be identified by probing,
5 a useful method for generating the 5' or 3' end is to use the existing sequence information
from the original cDNA to generate the missing information. A method similar to
5'RACE is available for generating the missing 5' end of a desired full-length gene. (This
method was published by Fromont-Racine et al., *Nucleic Acids Res.*, 21(7): 1683-1684
10 (1993)). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population
of RNA presumably 30 containing full-length gene RNA transcript and a primer set
containing a primer specific to the ligated RNA oligonucleotide and a primer specific to
a known sequence of the gene of interest, is used to PCR amplify the 5' portion of the
desired full length gene which may then be sequenced and used to generate the full length
15 gene. This method starts with total RNA isolated from the desired source, poly A RNA
may be used but is not a prerequisite for this procedure. The RNA preparation may then
be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or
damaged RNA which may interfere with the later RNA ligase step. The phosphatase if
used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in
20 order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction
leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be
ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation
can then be used as a template for first strand cDNA synthesis using a gene specific
oligonucleotide. The first strand synthesis reaction can then be used as a template for
25 PCR amplification of the desired 5' end using a primer specific to the ligated RNA
oligonucleotide and a primer specific to the known sequence of the apoptosis related of
interest. The resultant product is then sequenced and analyzed to confirm that the 5' end
sequence belongs to the relevant apoptosis related.

30

Example 21 - Tissue Distribution Of Polypeptide.

Tissue distribution of mRNA expression of polynucleotides of the present
invention is determined using protocols for Northern blot analysis, described by, among
35 others, Sambrook et al. For example, a cDNA probe produced by the method described
in Example 20 is labeled with p32 using the rediprime(tm) DNA labeling system
(Amersham Life Science), according to manufacturer's instructions. After labeling, the

probe is purified using CHROMA SPIN0-100 column (Clontech Laboratories, Inc.)
5 according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various tissues for mRNA expression.

Tissue Northern blots containing the bound mRNA of various tissues are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech according to manufacturers protocol number PT1190-1. Northern blots can be produced
10 using various protocols well known in the art (e.g., Sambrook et al). Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and the films developed according to standard procedures.

15 **Example 22 - Chromosomal Mapping Of The Polynucleotides.**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:1 or SEQ ID NO:13. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions: 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This
20 cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Mammalian DNA, preferably human DNA, is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions are analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels.
25 Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 23 - Bacterial Expression Of A Polypeptide.

A polynucleotide encoding a polypeptide of the present invention is amplified
30 using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 20, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme
35 sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Ampr), a bacterial origin of replication (ori),

an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-
5 histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, that expresses the lacI
10 repressor and also confers kanamycin resistance (Kanr). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid
15 culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.600) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

20 Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc.,
25 supra). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., supra).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8,
30 the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the
35 protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors.

The renaturation should be performed over a period of 1.5 hours or more. After
5 renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

10 **Example 24 - Purification Of A Polypeptide From An Inclusion Body.**

The following alternative method can be used to purify a polypeptide expressed in E coli when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the E. coli fermentation, the cell
15 culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high
20 shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by
25 centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C
30 overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by
35 vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g.,

Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered
5 sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perceptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

10 Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perceptive Biosystems) and weak anion (Poros CM-20, Perceptive Biosystems) exchange resins. The columns are equilibrated with 40
15 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A280 monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-
20 PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Coomassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The
25 purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 25 - Cloning And Expression Of A Polypeptide In A Baculovirus Expression System.

30 In this example, the plasmid shuttle vector pAc373 is used to insert a polynucleotide into a baculovirus to express a polypeptide. A typical baculovirus expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites, which may include, for example BamHI, Xba I and Asp718. The polyadenylation site of the
35 simian virus 40 ("SV40") is often used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from E. coli under control of a weak Drosophila promoter in the same orientation, followed by the

polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both
5 sides by viral sequences for cell-mediated homologous recombination with wild-type
viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as
pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the
construct provides appropriately located signals for transcription, translation, secretion
10 and the like, including a signal peptide and an in-frame AUG as required. Such vectors
are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

A polynucleotide encoding a polypeptide of the present invention is amplified
using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA
15 sequence, as outlined in Example 20, to synthesize insertion fragments. The primers used
to amplify the cDNA insert should preferably contain restriction sites at the 5' end of the
primers in order to clone the amplified product into the expression vector. Specifically,
the cDNA sequence contained in the deposited clone, including the AUG initiation codon
and the naturally associated leader sequence identified elsewhere herein (if applicable),
20 is amplified using the PCR protocol described in Example 20. If the naturally occurring
signal sequence is used to produce the protein, the vector used does not need a second
signal peptide. Alternatively, the vector can be modified to include a baculovirus leader
sequence, using the standard methods described in Summers et al., "A Manual of
Methods for Baculovirus Vectors and Insect Cell Culture Procedures" Texas Agricultural
25 Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially
available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.). The fragment then is digested
with appropriate restriction enzymes and again purified on a 1% agarose gel.

30 The plasmid is digested with the corresponding restriction enzymes and
optionally, can be dephosphorylated using calf intestinal phosphatase, using routine
procedures known in the art. The DNA is then isolated from a 1% agarose gel using a
commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4
35 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene
Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and
spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA

from individual colonies and analyzing the digestion product by gel electrophoresis. The
5 sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transformed with 1.0 ug
of a commercially available linearized baculovirus DNA ("BaculoGoldtm baculovirus
DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner
et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGoldtm virus
10 DNA and 5ug of the plasmid are mixed in a sterile well of a microtiter plate containing
50ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD).
Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated
for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to
15 Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml
Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C.
The transfection solution is then removed from the plate and 1 ml of Grace's insect
medium supplemented with 10% fetal calf serum is added. Cultivation is then continued
at 27 degrees C for four days.

20 After four days the supernatant is collected and a plaque assay is performed, as
described by Summers and Smith, supra. An agarose gel with "Blue Gal" (Life
Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-
expressing clones, which produce blue-stained plaques. (A detailed description of a
25 "plaque assay" of this type can also be found in the user's guide for insect cell culture and
baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After
appropriate incubation, blue stained plaques are picked with the tip of a micropipettor
(e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a
microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing
30 the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days
later the supernatants of these culture dishes are harvested and then they are stored at 4
degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's
medium supplemented with 10% heat-inactivated FBS. The cells are infected with the
35 recombinant baculovirus containing the polynucleotide at a multiplicity of infection
("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is
removed and is replaced with SF900 II medium minus methionine and cysteine (available

from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of 35S-methionine
5 and 5 uCi 35S-cysteine (available from Amersham) are added. The cells are further
incubated for 16 hours and then are harvested by centrifugation. The proteins in the
supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by
autoradiography (if radiolabeled).

10 Microsequencing of the amino acid sequence of the amino terminus of purified
protein may be used to determine the amino terminal sequence of the produced protein.

Example 26 - Expression Of A Polypeptide In Mammalian Cells.

The polypeptide of the present invention can be expressed in a mammalian cell.
15 A typical mammalian expression vector contains a promoter element, which mediates the
initiation of transcription of mRNA, a protein coding sequence, and signals required for
the termination of transcription and polyadenylation of the transcript. Additional elements
include enhancers, Kozak sequences and intervening sequences flanked by donor and
acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early
20 and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g.,
RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However,
cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for
example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat
25 (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0,
and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293,
H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3
cells, mouse L cells and Chinese hamster ovary (CHO) cells.

30 Alternatively, the polypeptide can be expressed in stable cell lines containing the
polynucleotide integrated into a chromosome. The co-transformation with a selectable
marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation
of the transformed cells.

35 The transformed gene can also be amplified to express large amounts of the
encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell
lines that carry several hundred or even several thousand copies of the gene of interest.
(See, e.g., Alt, F. W., et al., J. Biol. Chem., 253:1357-1370 (1978); Hamlin, J. L. and Ma,

C., *Biochem. et Biophys. Acta*, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A.,
5 *Biotechnology* 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine
synthase (GS) (Murphy et al., *Biochem J.* 227:277-279 (1991); Bebbington et al.,
Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown
in selective medium and the cells with the highest resistance are selected. These cell lines
10 contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary
(CHO) and NSO cells are often used for the production of proteins.

A polynucleotide of the present invention is amplified according to the protocol
outlined in herein. If the naturally occurring signal sequence is used to produce the
protein, the vector does not need a second signal peptide. Alternatively, if the naturally
15 occurring signal sequence is not used, the vector can be modified to include a
heterologous signal sequence. (See, e.g., WO 96/34891.) The amplified fragment is
isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101
Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes
and again purified on a 1% agarose gel.

20 The amplified fragment is then digested with the same restriction enzyme and
purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are
then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed
and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for
25 instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for
transformation. Five µg of an expression plasmid is cotransformed with 0.5 ug of the
plasmid pSVneo using lipofectin (Felgner et al., supra). The plasmid pSV2-neo contains
a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers
30 resistance to a group of antibiotics including G418. The cells are seeded in alpha minus
MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and
seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM
supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about
10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml
35 flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM,
800 nM). Clones growing at the highest concentrations of methotrexate are then
transferred to new 6-well plates containing even higher concentrations of methotrexate

(1 uM, 2 uM, 5 uM, 10 mM, 20 mM). The same procedure is repeated until clones are
5 obtained which grow at a concentration of 100 - 200 uM. Expression of the desired gene
product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase
HPLC analysis.

10 **Example 27 – Method of Creating N- and C-terminal Deletion Mutants
Corresponding to the HGPRBMY1 and HGPRBMY2 Polypeptides of the Present
Invention.**

As described elsewhere herein, the present invention encompasses the creation
of N- and C-terminal deletion mutants, in addition to any combination of N- and C-
15 terminal deletions thereof, corresponding to the HGPRBMY1 or HGPRBMY2
polypeptide of the present invention. A number of methods are available to one skilled
in the art for creating such mutants. Such methods may include a combination of PCR
amplification and gene cloning methodology. Although one of skill in the art of
molecular biology, through the use of the teachings provided or referenced herein, and/or
20 otherwise known in the art as standard methods, could readily create each deletion mutant
of the present invention, exemplary methods are described below.

Briefly, using the isolated cDNA clone encoding the full-length HGPRBMY1 or
HGPRBMY2 polypeptide sequence (as described herein, for example), appropriate
25 primers of about 15-25 nucleotides derived from the desired 5' and 3' positions of SEQ
ID NO:1 or SEQ ID NO:13 may be designed to PCR amplify, and subsequently clone,
the intended N- and/or C-terminal deletion mutant. Such primers could comprise, for
example, an initiation and stop codon for the 5' and 3' primer, respectively. Such
primers may also comprise restriction sites to facilitate cloning of the deletion mutant
30 post amplification. Moreover, the primers may comprise additional sequences, such as,
for example, flag-tag sequences, kozac sequences, or other sequences discussed and/or
referenced herein.

For example, in the case of the M76 to H431 HGPRBMY2 N-terminal deletion
mutant, the following primers could be used to amplify a cDNA fragment corresponding
35 to this deletion mutant:

5	5' Primer	5'-GCAGCA <u>GCGGCCGC</u> ATGCGCACCGTCACCAACATC -3' (SEQ ID NO:20) <i>NotI</i>
10	3' Primer	5'- GCAGCA <u>GTCGAC</u> ATGCCCACTGTCTAAAGGAGAATTC -3' (SEQ ID NO:21) <i>Sall</i>

For example, in the case of the M1 to Y305 HGPRBMY2 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

15	5' Primer	5'- GCAGCA <u>GCGGCCGC</u> CGGCGCATGGGGCCCAGATCCCCG -3' (SEQ ID NO:22) <i>NotI</i>
20	3' Primer	5'- GCAGCA <u>GTCGAC</u> GAACACACTCTCCTGCCTCTGGAGG -3' (SEQ ID NO:23) <i>Sall</i>

For example, in the case of the R50 to F359 HGPRBMY1 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

30	5' Primer	5'-GCAGCA <u>GCGGCCGC</u> ATGCAGGTCCCGAACAGCACCGGCC -3' (SEQ ID NO:52) <i>NotI</i>
35	3' Primer	5'- GCAGCA <u>GTCGAC</u> CTTGTACACGTGGTAGTAGCTCTTG -3' (SEQ ID NO:53) <i>Sall</i>

For example, in the case of the M1 to K276 HGPRBMY1 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5	5' Primer	5'- GCAGCA <u>GCGGCCGC</u> ATGCAGGCGCTTAACATTACCCCGG -3' (SEQ ID NO:54) <i>NotI</i>
10	3' Primer	5'- GCAGCA <u>GTCGAC</u> ATATTCCTTTTCAAAATTACTG -3' (SEQ ID NO:55) <i>SalI</i>

Representative PCR amplification conditions are provided below, although the skilled artisan would appreciate that other conditions may be required for efficient amplification. A 100 ul PCR reaction mixture may be prepared using 10ng of the template DNA (cDNA clone of HGPRBMY2), 200 uM 4dNTPs, 1uM primers, 0.25U Taq DNA polymerase (PE), and standard Taq DNA polymerase buffer. Typical PCR cycling condition are as follows:

20-25 cycles: 45 sec, 93 degrees
2 min, 50 degrees
2 min, 72 degrees
1 cycle: 10 min, 72 degrees

After the final extension step of PCR, 5U Klenow Fragment may be added and incubated for 15 min at 30 degrees.

Upon digestion of the fragment with the NotI and SalI restriction enzymes, the fragment could be cloned into an appropriate expression and/or cloning vector which has been similarly digested (e.g., pSport1, among others). The skilled artisan would appreciate that other plasmids could be equally substituted, and may be desirable in certain circumstances. The digested fragment and vector are then ligated using a DNA ligase, and then used to transform competent E.coli cells using methods provided herein and/or otherwise known in the art.

The 5' primer sequence for amplifying any additional N-terminal deletion mutants may be determined by reference to the following formula:

(S+(X * 3)) to ((S+(X * 3))+25), wherein 'S' is equal to the nucleotide position
5 of the initiating start codon of the HGPRBMY1 or HGPRBMY2 gene (SEQ ID NO:1 or
SEQ ID NO:13), and 'X' is equal to the most N-terminal amino acid of the intended N-
terminal deletion mutant. The first term will provide the start 5' nucleotide position of
the 5' primer, while the second term will provide the end 3' nucleotide position of the 5'
10 primer corresponding to sense strand of SEQ ID NO:1 or SEQ ID NO:13 . Once the
corresponding nucleotide positions of the primer are determined, the final nucleotide
sequence may be created by the addition of applicable restriction site sequences to the 5'
end of the sequence, for example. As referenced herein, the addition of other sequences
to the 5' primer may be desired in certain circumstances (e.g., kozac sequences, etc.).

15 The 3' primer sequence for amplifying any additional N-terminal deletion
mutants may be determined by reference to the following formula:

(S+(X * 3)) to ((S+(X * 3))-25), wherein 'S' is equal to the nucleotide position
of the initiating start codon of the HGPRBMY1 or HGPRBMY2 gene (SEQ ID NO:1 or
SEQ ID NO:13), and 'X' is equal to the most C-terminal amino acid of the intended N-
20 terminal deletion mutant. The first term will provide the start 5' nucleotide position of
the 3' primer, while the second term will provide the end 3' nucleotide position of the 3'
primer corresponding to the anti-sense strand of SEQ ID NO:1 or SEQ ID NO:13 . Once
the corresponding nucleotide positions of the primer are determined, the final nucleotide
25 sequence may be created by the addition of applicable restriction site sequences to the 5'
end of the sequence, for example. As referenced herein, the addition of other sequences
to the 3' primer may be desired in certain circumstances (e.g., stop codon sequences,
etc.). The skilled artisan would appreciate that modifications of the above nucleotide
positions may be necessary for optimizing PCR amplification.

30 The same general formulas provided above may be used in identifying the 5' and
3' primer sequences for amplifying any C-terminal deletion mutant of the present
invention. Moreover, the same general formulas provided above may be used in
identifying the 5' and 3' primer sequences for amplifying any combination of N-terminal
and C-terminal deletion mutant of the present invention. The skilled artisan would
35 appreciate that modifications of the above nucleotide positions may be necessary for
optimizing PCR amplification.

5 **Example 28 - Protein Fusions.**

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example described herein; see also EP A 394,827; 10 Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the 15 activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

20 Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be 25 produced.

The naturally occurring signal sequence may be used to produce the protein (if applicable). Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 30 96/34891 and/or US Patent No. 6,066,781, supra.)

Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCCACCGT
GCCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAAA
35 CCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGG
TGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAG

CACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAAT
5 GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAACCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACA
CCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTG
CCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAAT
10 GGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG
GCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA
GGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC
ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGCGAC
TCTAGAGGAT (SEQ ID NO:24)

15

Example 29 - Production Of An Antibody From A Polypeptide.

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention are administered to an animal to induce the
20 production of sera containing polyclonal antibodies. In a preferred method, a preparation of the protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

25 In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium
35 supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma
5 cell line. Any suitable myeloma cell line may be employed in accordance with the present
invention; however, it is preferable to employ the parent myeloma cell line (SP2O),
available from the ATCC. After fusion, the resulting hybridoma cells are selectively
maintained in HAT medium, and then cloned by limiting dilution as described by Wands
et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such
10 a selection are then assayed to identify clones which secrete antibodies capable of binding
the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be
produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes
15 use of the fact that antibodies are themselves antigens, and therefore, it is possible to
obtain an antibody that binds to a second antibody. In accordance with this method,
protein specific antibodies are used to immunize an animal, preferably a mouse. The
splenocytes of such an animal are then used to produce hybridoma cells, and the
hybridoma cells are screened to identify clones that produce an antibody whose ability
20 to bind to the protein-specific antibody can be blocked by the polypeptide. Such
antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be
used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies
25 of the present invention may be used according to the methods disclosed herein. Such
fragments are typically produced by proteolytic cleavage, using enzymes such as papain
(to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). Alternatively,
protein-binding fragments can be produced through the application of recombinant DNA
technology or through synthetic chemistry.

30 For in vivo use of antibodies in humans, it may be preferable to use "humanized"
chimeric monoclonal antibodies. Such antibodies can be produced using genetic
constructs derived from hybridoma cells producing the monoclonal antibodies described
above. Methods for producing chimeric antibodies are known in the art. (See, for review,
Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et
35 al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494;
Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature
312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Moreover, in another preferred method, the antibodies directed against the polypeptides of the present invention may be produced in plants. Specific methods are disclosed in US Patent Nos. 5,959,177, and 6,080,560, which are hereby incorporated in their entirety herein. The methods not only describe methods of expressing antibodies, but also the means of assembling foreign multimeric proteins in plants (i.e., antibodies, etc.), and the subsequent secretion of such antibodies from the plant.

Example 26 - Method Of Enhancing The Biological Activity/Functional Characteristics Of Invention Through Molecular Evolution.

Although many of the most biologically active proteins known are highly effective for their specified function in an organism, they often possess characteristics that make them undesirable for transgenic, therapeutic, and/or industrial applications. Among these traits, a short physiological half-life is the most prominent problem, and is present either at the level of the protein, or the level of the proteins mRNA. The ability to extend the half-life, for example, would be particularly important for a proteins use in gene therapy, transgenic animal production, the bioprocess production and purification of the protein, and use of the protein as a chemical modulator among others. Therefore, there is a need to identify novel variants of isolated proteins possessing characteristics which enhance their application as a therapeutic for treating diseases of animal origin, in addition to the proteins applicability to common industrial and pharmaceutical applications.

Thus, one aspect of the present invention relates to the ability to enhance specific characteristics of invention through directed molecular evolution. Such an enhancement may, in a non-limiting example, benefit the inventions utility as an essential component in a kit, the inventions physical attributes such as its solubility, structure, or codon optimization, the inventions specific biological activity, including any associated enzymatic activity, the proteins enzyme kinetics, the proteins K_i , K_{cat} , K_m , V_{max} , K_d , protein-protein activity, protein-DNA binding activity, antagonist/inhibitory activity (including direct or indirect interaction), agonist activity (including direct or indirect interaction), the proteins antigenicity (e.g., where it would be desirable to either increase or decrease the antigenic potential of the protein), the immunogenicity of the protein, the ability of the protein to form dimers, trimers, or multimers with either itself or other

proteins, the antigenic efficacy of the invention, including its subsequent use a
5 preventative treatment for disease or disease states, or as an effector for targeting diseased
genes. Moreover, the ability to enhance specific characteristics of a protein may also be
applicable to changing the characterized activity of an enzyme to an activity completely
unrelated to its initially characterized activity. Other desirable enhancements of the
10 invention would be specific to each individual protein, and would thus be well known in
the art and contemplated by the present invention.

For example, an engineered G-protein coupled receptor may be constitutively
active upon binding of its cognate ligand. Alternatively, an engineered G-protein coupled
receptor may be constitutively active in the absence of ligand binding. In yet another
15 example, an engineered GPCR may be capable of being activated with less than all of the
regulatory factors and/or conditions typically required for GPCR activation (e.g., ligand
binding, phosphorylation, conformational changes, etc.). Such GPCRs would be useful
in screens to identify GPCR modulators, among other uses described herein.

Directed evolution is comprised of several steps. The first step is to establish a
20 library of variants for the gene or protein of interest. The most important step is to then
select for those variants that entail the activity you wish to identify. The design of the
screen is essential since your screen should be selective enough to eliminate non-useful
variants, but not so stringent as to eliminate all variants. The last step is then to repeat the
25 above steps using the best variant from the previous screen. Each successive cycle, can
then be tailored as necessary, such as increasing the stringency of the screen, for example.

Over the years, there have been a number of methods developed to introduce
mutations into macromolecules. Some of these methods include, random mutagenesis,
"error-prone" PCR, chemical mutagenesis, site-directed mutagenesis, and other methods
30 well known in the art (for a comprehensive listing of current mutagenesis methods, see
Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold
Spring, NY (1982)). Typically, such methods have been used, for example, as tools for
identifying the core functional region(s) of a protein or the function of specific domains
of a protein (if a multi-domain protein). However, such methods have more recently been
35 applied to the identification of macromolecule variants with specific or enhanced
characteristics.

Random mutagenesis has been the most widely recognized method to date.

Typically, this has been carried out either through the use of "error-prone" PCR (as
5 described in Moore, J., et al, Nature Biotechnology 14:458, (1996), or through the
application of randomized synthetic oligonucleotides corresponding to specific regions
of interest (as described by Derbyshire, K.M. et al, Gene, 46:145-152, (1986), and Hill,
DE, et al, Methods Enzymol., 55:559-568, (1987). Both approaches have limits to the
10 level of mutagenesis that can be obtained. However, either approach enables the
investigator to effectively control the rate of mutagenesis. This is particularly important
considering the fact that mutations beneficial to the activity of the enzyme are fairly rare.
In fact, using too high a level of mutagenesis may counter or inhibit the desired benefit
of a useful mutation.

15 While both of the aforementioned methods are effective for creating randomized
pools of macromolecule variants, a third method, termed "DNA Shuffling", or "sexual
PCR" (WPC, Stemmer, PNAS, 91:10747, (1994)) has recently been elucidated. DNA
shuffling has also been referred to as "directed molecular evolution", "exon-shuffling",
"directed enzyme evolution", "in vitro evolution", and "artificial evolution". Such
20 reference terms are known in the art and are encompassed by the invention. This new,
preferred, method apparently overcomes the limitations of the previous methods in that
it not only propagates positive traits, but simultaneously eliminates negative traits in the
resulting progeny.

25 DNA shuffling accomplishes this task by combining the principal of in vitro
recombination, along with the method of "error-prone" PCR. In effect, you begin with
a randomly digested pool of small fragments of your gene, created by Dnase I digestion,
and then introduce said random fragments into an "error-prone" PCR assembly reaction.
During the PCR reaction, the randomly sized DNA fragments not only hybridize to their
30 cognate strand, but also may hybridize to other DNA fragments corresponding to
different regions of the polynucleotide of interest – regions not typically accessible via
hybridization of the entire polynucleotide. Moreover, since the PCR assembly reaction
utilizes "error-prone" PCR reaction conditions, random mutations are introduced during
the DNA synthesis step of the PCR reaction for all of the fragments -further diversifying
35 the potential hybridization sites during the annealing step of the reaction.

A variety of reaction conditions could be utilized to carry-out the DNA shuffling
reaction. However, specific reaction conditions for DNA shuffling are provided, for

example, in PNAS, 91:10747, (1994). Briefly:

5 Prepare the DNA substrate to be subjected to the DNA shuffling reaction. Preparation may be in the form of simply purifying the DNA from contaminating cellular material, chemicals, buffers, oligonucleotide primers, deoxynucleotides, RNAs, etc., and may entail the use of DNA purification kits as those provided by Qiagen, Inc., or by the Promega, Corp., for example.

10 Once the DNA substrate has been purified, it would be subjected to Dnase I digestion. About 2-4ug of the DNA substrate(s) would be digested with .0015 units of Dnase I (Sigma) per ul in 100ul of 50mM Tris-HCL, pH 7.4/1mM MgCl₂ for 10-20 min. at room temperature. The resulting fragments of 10-50bp could then be purified by
15 running them through a 2% low-melting point agarose gel by electrophoresis onto DE81 ion-exchange paper (Whatmann) or could be purified using Microcon concentrators (Amicon) of the appropriate molecular weight cutoff, or could use oligonucleotide purification columns (Qiagen), in addition to other methods known in the art. If using DE81 ion-exchange paper, the 10-50bp fragments could be eluted from said paper using
20 1M NaCl, followed by ethanol precipitation.

The resulting purified fragments would then be subjected to a PCR assembly reaction by re-suspension in a PCR mixture containing: 2mM of each dNTP, 2.2mM MgCl₂, 50 mM KCl, 10mM Tris•HCL, pH 9.0, and 0.1% Triton X-100, at a final
25 fragment concentration of 10-30ng/ul. No primers are added at this point. *Taq* DNA polymerase (Promega) would be used at 2.5 units per 100ul of reaction mixture. A PCR program of 94 C for 60s; 94 C for 30s, 50-55 C for 30s, and 72 C for 30s using 30-45 cycles, followed by 72 C for 5min using an MJ Research (Cambridge, MA) PTC-150 thermocycler. After the assembly reaction is completed, a 1:40 dilution of the resulting
30 primerless product would then be introduced into a PCR mixture (using the same buffer mixture used for the assembly reaction) containing 0.8um of each primer and subjecting this mixture to 15 cycles of PCR (using 94 C for 30s, 50 C for 30s, and 72 C for 30s). The referred primers would be primers corresponding to the nucleic acid sequences of the polynucleotide(s) utilized in the shuffling reaction. Said primers could consist of
35 modified nucleic acid base pairs using methods known in the art and referred to elsewhere herein, or could contain additional sequences (i.e., for adding restriction sites, mutating specific base-pairs, etc.).

The resulting shuffled, assembled, and amplified product can be purified using
5 methods well known in the art (e.g., Qiagen PCR purification kits) and then subsequently
cloned using appropriate restriction enzymes.

Although a number of variations of DNA shuffling have been published to date,
such variations would be obvious to the skilled artisan and are encompassed by the
invention. The DNA shuffling method can also be tailored to the desired level of
10 mutagenesis using the methods described by Zhao, et al. (Nucl Acid Res., 25(6):1307-
1308, (1997)).

As described above, once the randomized pool has been created, it can then be
subjected to a specific screen to identify the variant possessing the desired
15 characteristic(s). Once the variant has been identified, DNA corresponding to the variant
could then be used as the DNA substrate for initiating another round of DNA shuffling.
This cycle of shuffling, selecting the optimized variant of interest, and then re-shuffling,
can be repeated until the ultimate variant is obtained. Examples of model screens applied
to identify variants created using DNA shuffling technology may be found in the
20 following publications: J. C., Moore, et al., J. Mol. Biol., 272:336-347, (1997), F.R.,
Cross, et al., Mol. Cell. Biol., 18:2923-2931, (1998), and A. Cramer, et al., Nat.
Biotech., 15:436-438, (1997).

DNA shuffling has several advantages. First, it makes use of beneficial mutations.
25 When combined with screening, DNA shuffling allows the discovery of the best
mutational combinations and does not assume that the best combination contains all the
mutations in a population. Secondly, recombination occurs simultaneously with point
mutagenesis. An effect of forcing DNA polymerase to synthesize full-length genes from
the small fragment DNA pool is a background mutagenesis rate. In combination with a
30 stringent selection method, enzymatic activity has been evolved up to 16000 fold increase
over the wild-type form of the enzyme. In essence, the background mutagenesis yielded
the genetic variability on which recombination acted to enhance the activity.

A third feature of recombination is that it can be used to remove deleterious
mutations. As discussed above, during the process of the randomization, for every one
35 beneficial mutation, there may be at least one or more neutral or inhibitory mutations.
Such mutations can be removed by including in the assembly reaction an excess of the
wild-type random-size fragments, in addition to the random-size fragments of the

selected mutant from the previous selection. During the next selection, some of the most
5 active variants of the polynucleotide/polypeptide/enzyme, should have lost the inhibitory
mutations.

Finally, recombination enables parallel processing. This represents a significant
advantage since there are likely multiple characteristics that would make a protein more
desirable (e.g. solubility, activity, etc.). Since it is increasingly difficult to screen for more
10 than one desirable trait at a time, other methods of molecular evolution tend to be
inhibitory. However, using recombination, it would be possible to combine the
randomized fragments of the best representative variants for the various traits, and then
select for multiple properties at once.

15 DNA shuffling can also be applied to the polynucleotides and polypeptides of the
present invention to decrease their immunogenicity in a specified host. For example, a
particular variant of the present invention may be created and isolated using DNA
shuffling technology. Such a variant may have all of the desired characteristics, though
may be highly immunogenic in a host due to its novel intrinsic structure. Specifically, the
20 desired characteristic may cause the polypeptide to have a non-native structure which
could no longer be recognized as a "self" molecule, but rather as a "foreign", and thus
activate a host immune response directed against the novel variant. Such a limitation can
be overcome, for example, by including a copy of the gene sequence for a xenobiotic
ortholog of the native protein in with the gene sequence of the novel variant gene in one
25 or more cycles of DNA shuffling. The molar ratio of the ortholog and novel variant
DNAs could be varied accordingly. Ideally, the resulting hybrid variant identified would
contain at least some of the coding sequence which enabled the xenobiotic protein to
evade the host immune system, and additionally, the coding sequence of the original
30 novel variant that provided the desired characteristics.

Likewise, the invention encompasses the application of DNA shuffling
technology to the evolution of polynucleotides and polypeptides of the invention, wherein
one or more cycles of DNA shuffling include, in addition to the gene template DNA,
oligonucleotides coding for known allelic sequences, optimized codon sequences, known
35 variant sequences, known polynucleotide polymorphism sequences, known ortholog
sequences, known homologue sequences, additional homologous sequences, additional
non-homologous sequences, sequences from another species, and any number and

combination of the above.

5 In addition to the described methods above, there are a number of related methods that may also be applicable, or desirable in certain cases. Representative among these are the methods discussed in PCT applications WO 98/31700, and WO 98/32845, which are hereby incorporated by reference. Furthermore, related methods can also be applied to the polynucleotide sequences of the present invention in order to evolve invention for
10 creating ideal variants for use in gene therapy, protein engineering, evolution of whole cells containing the variant, or in the evolution of entire enzyme pathways containing polynucleotides of the invention as described in PCT applications WO 98/13485, WO 98/13487, WO 98/27230, WO 98/31837, and Crameri, A., et al., Nat. Biotech., 15:436-
15 438, (1997), respectively.

Additional methods of applying "DNA Shuffling" technology to the polynucleotides and polypeptides of the present invention, including their proposed applications, may be found in US Patent No. 5,605,793; PCT Application No. WO 95/22625; PCT Application No. WO 97/20078; PCT Application No. WO 97/35966; and
20 PCT Application No. WO 98/42832; PCT Application No. WO 00/09727 specifically provides methods for applying DNA shuffling to the identification of herbicide selective crops which could be applied to the polynucleotides and polypeptides of the present invention; additionally, PCT Application No. WO 00/12680 provides methods and
25 compositions for generating, modifying, adapting, and optimizing polynucleotide sequences that confer detectable phenotypic properties on plant species; each of the above are hereby incorporated in their entirety herein for all purposes.

Example 30 - Method Of Determining Alterations In A Gene Corresponding To A
30 **Polynucleotide.**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in
35 SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4
5 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies).
The intron-exon borders of selected exons is also determined and genomic PCR products
analyzed to confirm the results. PCR products harboring suspected mutations is then
cloned and sequenced to validate the results of the direct sequencing.

10 PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic
Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States
Biochemical). Affected individuals are identified by mutations not present in unaffected
individuals.

Genomic rearrangements are also observed as a method of determining alterations
15 in a gene corresponding to a polynucleotide. Genomic clones isolated according to
Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer
Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-
99 (1991). Hybridization with the labeled probe is carried out using a vast excess of
human cot-1 DNA for specific hybridization to the corresponding genomic locus.

20 Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium
iodide, producing a combination of C- and R-bands. Aligned images for precise mapping
are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in
combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ)
25 and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75
(1991).) Image collection, analysis and chromosomal fractional length measurements are
performed using the ISee Graphical Program System. (Inovision Corporation, Durham,
NC.) Chromosome alterations of the genomic region hybridized by the probe are
identified as insertions, deletions, and translocations. These alterations are used as a
30 diagnostic marker for an associated disease.

Example 31 - Method Of Detecting Abnormal Levels Of A Polypeptide In A Biological Sample.

35 A polypeptide of the present invention can be detected in a biological sample, and
if an increased or decreased level of the polypeptide is detected, this polypeptide is a
marker for a particular phenotype. Methods of detection are numerous, and thus, it is
understood that one skilled in the art can modify the following assay to fit their particular

needs.

5 For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described elsewhere herein. The wells are blocked so that non-specific binding of the polypeptide to the well is
10 reduced.

 The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to
15 remove unbounded polypeptide.

 Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

20 Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and
25 fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 32 – Formulation.

 The invention also provides methods of treatment and/or prevention diseases,
30 disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile
35 carrier).

 The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient

(especially the side effects of treatment with the Therapeutic alone), the site of delivery,
5 the method of administration, the scheduling of administration, and other factors known
to practitioners. The "effective amount" for purposes herein is thus determined by such
considerations.

As a general proposition, the total pharmaceutically effective amount of the
Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day
10 to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to
therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most
preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given
continuously, the Therapeutic is typically administered at a dose rate of about 1
15 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous
subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution
may also be employed. The length of treatment needed to observe changes and the
interval following treatment for responses to occur appears to vary depending on the
desired effect.

20 Therapeutics can be administered orally, rectally, parenterally, intracisternally,
intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or
transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable
carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating
25 material or formulation auxiliary of any. The term "parenteral" as used herein refers to
modes of administration which include intravenous, intramuscular, intraperitoneal,
intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release
systems. Suitable examples of sustained-release Therapeutics are administered orally,
30 rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by
powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal
spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or
liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term
"parenteral" as used herein refers to modes of administration which include intravenous,
35 intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and
infusion.

Therapeutics of the invention may also be suitably administered by sustained-

release systems. Suitable examples of sustained-release Therapeutics include suitable
5 polymeric materials (such as, for example, semi-permeable polymer matrices in the form
of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for
example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly
soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP
10 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al.,
Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J.
Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)),
ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP
15 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics
of the invention (see, generally, Langer, Science 249:1527-1533 (1990); Treat et al., in
Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler
(eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the
20 Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc.
Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA)
77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641;
Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324.
25 Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type
in which the lipid content is greater than about 30 mol. percent cholesterol, the selected
proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered
by way of a pump (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201
30 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574
(1989)).

Other controlled release systems are discussed in the review by Langer (Science
249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated
35 generally by mixing it at the desired degree of purity, in a unit dosage injectable form
(solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one
that is non-toxic to recipients at the dosages and concentrations employed and is

compatible with other ingredients of the formulation. For example, the formulation
5 preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if
10 necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

15 The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine
20 or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium;
25 and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers
30 will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access
35 port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation

for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with
5 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture
is lyophilized. The infusion solution is prepared by reconstituting the lyophilized
Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more
containers filled with one or more of the ingredients of the Therapeutics of the invention.
10 Associated with such container(s) can be a notice in the form prescribed by a
governmental agency regulating the manufacture, use or sale of pharmaceuticals or
biological products, which notice reflects approval by the agency of manufacture, use or
sale for human administration. In addition, the Therapeutics may be employed in
15 conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination
with adjuvants. Adjuvants that may be administered with the Therapeutics of the
invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg),
MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific
20 embodiment, Therapeutics of the invention are administered in combination with alum.
In another specific embodiment, Therapeutics of the invention are administered in
combination with QS-21. Further adjuvants that may be administered with the
Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid
25 immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59,
and Virosomal adjuvant technology. Vaccines that may be administered with the
Therapeutics of the invention include, but are not limited to, vaccines directed toward
protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria,
hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia,
30 influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis,
poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered
either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently;
or sequentially. This includes presentations in which the combined agents are
administered together as a therapeutic mixture, and also procedures in which the
35 combined agents are administered separately but simultaneously, e.g., as through separate
intravenous lines into the same individual. Administration "in combination" further
includes the separate administration of one of the compounds or agents given first,

followed by the second.

5 The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or
10 growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately
15 but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

 In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules
20 that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International
25 Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB, TR2 (International Publication No. WO 96/34095), DR3
30 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International
35 Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

 In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-

nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse
5 transcriptase inhibitors that may be administered in combination with the Therapeutics
of the invention, include, but are not limited to, RETROVIR (zidovudine/AZT), VIDEX
(didanosine/ddI), HIVID (zalcitabine/ddC), ZERIT (stavudine/d4T), EPIVIR
(lamivudine/3TC), and COMBIVIR (zidovudine/lamivudine). Non-nucleoside reverse
10 transcriptase inhibitors that may be administered in combination with the Therapeutics
of the invention, include, but are not limited to, VIRAMUNE (nevirapine),
RESCRIPTOR (delavirdine), and SUSTIVA (efavirenz). Protease inhibitors that may be
administered in combination with the Therapeutics of the invention, include, but are not
limited to, CRIXIVAN (indinavir), NORVIR (ritonavir), INVIRASE (saquinavir), and
15 VIRACEPT (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside
reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or
protease inhibitors may be used in any combination with Therapeutics of the invention
to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in
20 combination with anti-opportunistic infection agents. Anti-opportunistic agents that may
be administered in combination with the Therapeutics of the invention, include, but are
not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE, DAPSONE,
PENTAMIDINE, ATOVAQUONE, ISONIAZID, RIFAMPIN, PYRAZINAMIDE,
25 ETHAMBUTOL, RIFABUTIN, CLARITHROMYCIN, AZITHROMYCIN,
GANCICLOVIR, FOSCARNET, CIDOFOVIR, FLUCONAZOLE, ITRACONAZOLE,
KETOCONAZOLE, ACYCLOVIR, FAMCICOLVIR, PYRIMETHAMINE,
LEUCOVORIN, NEUPOGEN (filgrastim/G-CSF), and LEUKINE (sargramostim/GM-
CSF). In a specific embodiment, Therapeutics of the invention are used in any
30 combination with TRIMETHOPRIM-SULFAMETHOXAZOLE, DAPSONE,
PENTAMIDINE, and/or ATOVAQUONE to prophylactically treat or prevent an
opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment,
Therapeutics of the invention are used in any combination with ISONIAZID, RIFAMPIN,
PYRAZINAMIDE, and/or ETHAMBUTOL to prophylactically treat or prevent an
35 opportunistic *Mycobacterium avium* complex infection. In another specific embodiment,
Therapeutics of the invention are used in any combination with RIFABUTIN,
CLARITHROMYCIN, and/or AZITHROMYCIN to prophylactically treat or prevent an

opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment,
5 Therapeutics of the invention are used in any combination with GANCICLOVIR,
FOSCARNET, and/or CIDOFOVIR to prophylactically treat or prevent an opportunistic
cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention
are used in any combination with FLUCONAZOLE, ITRACONAZOLE, and/or
10 KETOCONAZOLE to prophylactically treat or prevent an opportunistic fungal infection.
In another specific embodiment, Therapeutics of the invention are used in any
combination with ACYCLOVIR and/or FAMCICLOVIR to prophylactically treat or
prevent an opportunistic herpes simplex virus type I and/or type II infection. In another
specific embodiment, Therapeutics of the invention are used in any combination with
15 PYRIMETHAMINE and/or LEUCOVORIN to prophylactically treat or prevent an
opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics
of the invention are used in any combination with LEUCOVORIN and/or NEUPOGEN
to prophylactically treat or prevent an opportunistic bacterial infection.

20 In a further embodiment, the Therapeutics of the invention are administered in
combination with an antiviral agent. Antiviral agents that may be administered with the
Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin,
amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in
25 combination with an antibiotic agent. Antibiotic agents that may be administered with the
Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases,
aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin,
chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin,
fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin,
30 streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole,
and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered
in combination with the Therapeutics of the invention include, but are not limited to,
steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone,
35 prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive
agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in

combination with immunosuppressants. Immunosuppressants preparations that may be
5 administered with the Therapeutics of the invention include, but are not limited to,
ORTHOCLONE (OKT3), SANDIMMUNE/NEORAL/SANGDYA (cyclosporin),
PROGRAF (tacrolimus), CELLCEPT (mycophenolate), Azathioprine, glucocorticosteroids,
and RAPAMUNE (sirolimus). In a specific embodiment, immunosuppressants may be
10 used to prevent rejection of organ or bone marrow transplantation.

10 In an additional embodiment, Therapeutics of the invention are administered
alone or in combination with one or more intravenous immune globulin preparations.
Intravenous immune globulin preparations that may be administered with the
Therapeutics of the invention include, but not limited to, GAMMAR, IVEEGAM,
15 SANDOGLOBULIN, GAMMAGARD S/D, and GAMIMUNE. In a specific
embodiment, Therapeutics of the invention are administered in combination with
intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow
transplant).

20 In an additional embodiment, the Therapeutics of the invention are administered
alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that
may be administered with the Therapeutics of the invention include, but are not limited
to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid
derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids,
25 arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives,
thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-
hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide,
ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol,
paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

30 In another embodiment, compositions of the invention are administered in
combination with a chemotherapeutic agent. Chemotherapeutic agents that may be
administered with the Therapeutics of the invention include, but are not limited to,
antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin);
antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate,
35 floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-
thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine
arabioside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin,

busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone,
5 estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate,
methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone);
nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen
mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium
phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate,
10 vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in
combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)
or any combination of the components of CHOP. In another embodiment, Therapeutics
15 of the invention are administered in combination with Rituximab. In a further
embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP,
or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered
in combination with cytokines. Cytokines that may be administered with the Therapeutics
20 of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12,
IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment,
Therapeutics of the invention may be administered with any interleukin, including, but
not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10,
25 IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered
in combination with angiogenic proteins. Angiogenic proteins that may be administered
with the Therapeutics of the invention include, but are not limited to, Glioma Derived
Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet
30 Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-
682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent
Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International
Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in
Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor
35 (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular
Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-
506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International

Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3);
5 Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International
Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D),
as disclosed in International Publication Number WO 98/02543; Vascular Endothelial
Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO
98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German
10 Patent Number DE19639601. The above mentioned references are incorporated herein
by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered
in combination with hematopoietic growth factors. Hematopoietic growth factors that
15 may be administered with the Therapeutics of the invention include, but are not limited
to, LEUKINE (SARGRAMOSTIM) and NEUPOGEN (FILGRASTIM).

In an additional embodiment, the Therapeutics of the invention are administered
in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be
administered with the Therapeutics of the invention include, but are not limited to, FGF-
20 1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-
12, FGF-13, FGF-14, and FGF-15.

In a specific embodiment, formulations of the present invention may further
comprise antagonists of P-glycoprotein (also referred to as the multiresistance protein,
25 or PGP), including antagonists of its encoding polynucleotides (e.g., antisense
oligonucleotides, ribozymes, zinc-finger proteins, etc.). P-glycoprotein is well known for
decreasing the efficacy of various drug administrations due to its ability to export
intracellular levels of absorbed drug to the cell exterior. While this activity has been
particularly pronounced in cancer cells in response to the administration of chemotherapy
30 regimens, a variety of other cell types and the administration of other drug classes have
been noted (e.g., T-cells and anti-HIV drugs). In fact, certain mutations in the PGP gene
significantly reduces PGP function, making it less able to force drugs out of cells. People
who have two versions of the mutated gene--one inherited from each parent--have more
than four times less PGP than those with two normal versions of the gene. People may
35 also have one normal gene and one mutated one. Certain ethnic populations have
increased incidence of such PGP mutations. Among individuals from Ghana, Kenya, the
Sudan, as well as African Americans, frequency of the normal gene ranged from 73% to

84%. In contrast, the frequency was 34% to 59% among British whites, Portuguese,
5 Southwest Asian, Chinese, Filipino and Saudi populations. As a result, certain ethnic
populations may require increased administration of PGP antagonist in the formulation
of the present invention to arrive at the an efficacious dose of the therapeutic (e.g., those
from african descent). Conversely, certain ethnic populations, particularly those having
10 increased frequency of the mutated PGP (e.g., of caucasian descent, or non-african
descent) may require less pharmaceutical compositions in the formulation due to an
effective increase in efficacy of such compositions as a result of the increased effective
absorption (e.g., less PGP activity) of said composition.

In additional embodiments, the Therapeutics of the invention are administered in
15 combination with other therapeutic or prophylactic regimens, such as, for example,
radiation therapy.

Example 33 - Method Of Treating Decreased Levels Of The Polypeptide.

The present invention relates to a method for treating an individual in need of an
20 increased level of a polypeptide of the invention in the body comprising administering
to such an individual a composition comprising a therapeutically effective amount of an
agonist of the invention (including polypeptides of the invention). Moreover, it will be
appreciated that conditions caused by a decrease in the standard or normal expression
25 level of a secreted protein in an individual can be treated by administering the
polypeptide of the present invention, preferably in the secreted form. Thus, the invention
also provides a method of treatment of an individual in need of an increased level of the
polypeptide comprising administering to such an individual a Therapeutic comprising an
amount of the polypeptide to increase the activity level of the polypeptide in such an
30 individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose
0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is
in the secreted form. The exact details of the dosing scheme, based on administration and
35 formulation, are provided herein.

Example 34 - Method Of Treating Increased Levels Of The Polypeptide.

The present invention also relates to a method of treating an individual in need

of a decreased level of a polypeptide of the invention in the body comprising
5 administering to such an individual a composition comprising a therapeutically effective
amount of an antagonist of the invention (including polypeptides and antibodies of the
invention).

In one example, antisense technology is used to inhibit production of a
polypeptide of the present invention. This technology is one example of a method of
10 decreasing levels of a polypeptide, preferably a secreted form, due to a variety of
etiologies, such as cancer. For example, a patient diagnosed with abnormally increased
levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5,
1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest
15 period if the treatment was well tolerated. The formulation of the antisense
polynucleotide is provided herein.

Example 35 - Method Of Treatment Using Gene Therapy-Ex Vivo.

One method of gene therapy transplants fibroblasts, which are capable of
20 expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a
subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and
separated into small pieces. Small chunks of the tissue are placed on a wet surface of a
tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned
25 upside down, closed tight and left at room temperature over night. After 24 hours at room
temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of
the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and
streptomycin) is added. The flasks are then incubated at 37 degree C for approximately
one week.

30 At this time, fresh media is added and subsequently changed every several days.
After an additional two weeks in culture, a monolayer of fibroblasts emerge. The
monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long
terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and
35 HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is
fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using

PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in
5 Example 20 using primers and having appropriate restriction sites and initiation/stop
codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer
includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear
backbone and the amplified EcoRI and HindIII fragment are added together, in the
10 presence of T4 DNA ligase. The resulting mixture is maintained under conditions
appropriate for ligation of the two fragments. The ligation mixture is then used to
transform bacteria HB101, which are then plated onto agar containing kanamycin for the
purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture
15 to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf
serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then
added to the media and the packaging cells transduced with the vector. The packaging
cells now produce infectious viral particles containing the gene (the packaging cells are
now referred to as producer cells).

20 Fresh media is added to the transduced producer cells, and subsequently, the
media is harvested from a 10 cm plate of confluent producer cells. The spent media,
containing the infectious viral particles, is filtered through a millipore filter to remove
detached producer cells and this media is then used to infect fibroblast cells. Media is
25 removed from a sub-confluent plate of fibroblasts and quickly replaced with the media
from the producer cells. This media is removed and replaced with fresh media. If the titer
of virus is high, then virtually all fibroblasts will be infected and no selection is required.
If the titer is very low, then it is necessary to use a retroviral vector that has a selectable
marker, such as neo or his. Once the fibroblasts have been efficiently infected, the
30 fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after
having been grown to confluence on cytodex 3 microcarrier beads.

35 **Example 36 - Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides Of The Invention.**

Another method of gene therapy according to the present invention involves
operably associating the endogenous polynucleotide sequence of the invention with a

promoter via homologous recombination as described, for example, in U.S. Patent NO:
5 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published
September 26, 1996; International Publication NO: WO 94/12650, published August 4,
1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al.,
Nature, 342:435-438 (1989). This method involves the activation of a gene which is
10 present in the target cells, but which is not expressed in the cells, or is expressed at a
lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting
sequences, which are homologous to the 5' non-coding sequence of endogenous
polynucleotide sequence, flanking the promoter. The targeting sequence will be
15 sufficiently near the 5' end of the polynucleotide sequence so the promoter will be
operably linked to the endogenous sequence upon homologous recombination. The
promoter and the targeting sequences can be amplified using PCR. Preferably, the
amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends.
Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme
20 site as the 5' end of the amplified promoter and the 5' end of the second targeting
sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with
the appropriate restriction enzymes and subsequently treated with calf intestinal
25 phosphatase. The digested promoter and digested targeting sequences are added together
in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions
appropriate for ligation of the two fragments. The construct is size fractionated on an
agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked
30 polynucleotides via electroporation. However, the polynucleotide constructs may also be
administered with transfection-facilitating agents, such as liposomes, viral sequences,
viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which
results in the promoter being operably linked to the endogenous polynucleotide sequence.
35 This results in the expression of polynucleotide corresponding to the polynucleotide in
the cell. Expression may be detected by immunological staining, or any other method
known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂ HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10⁶ cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5X10⁶ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The

cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf
5 serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is
aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after
having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now
produce the protein product. The fibroblasts can then be introduced into a patient as
10 described above.

Example 37 - Method Of Treatment Using Gene Therapy - In Vivo.

Another aspect of the present invention is using in vivo gene therapy methods to
15 treat disorders, diseases and conditions. The gene therapy method relates to the
introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences
into an animal to increase or decrease the expression of the polypeptide. The
polynucleotide of the present invention may be operatively linked to a promoter or any
other genetic elements necessary for the expression of the polypeptide by the target tissue.
20 Such gene therapy and delivery techniques and methods are known in the art, see, for
example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859;
Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res.
35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et
al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996)
25 (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers
injectable materials to the cells of an animal, such as, injection into the interstitial space
of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide
30 constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free
from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell,
including viral sequences, viral particles, liposome formulations, lipofectin or
precipitating agents and the like. However, the polynucleotides of the present invention
35 may also be delivered in liposome formulations (such as those taught in Felgner P.L. et
al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell
85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are
5 preferably constructs that will not integrate into the host genome nor will they contain
sequences that allow for replication. Any strong promoter known to those skilled in the
art can be used for driving the expression of DNA. Unlike other gene therapies
techniques, one major advantage of introducing naked nucleic acid sequences into target
10 cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have
shown that non-replicating DNA sequences can be introduced into cells to provide
production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues
within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow,
15 thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach,
intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue.
Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide
matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or
chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue
20 ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by
the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to
the interstitial space of muscle tissue is preferred for the reasons discussed below. They
may be conveniently delivered by injection into the tissues comprising these cells. They
25 are preferably delivered to and expressed in persistent, non-dividing cells which are
differentiated, although delivery and expression may be achieved in non-differentiated
or less completely differentiated cells, such as, for example, stem cells of blood or skin
fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and
express polynucleotides.

30 For the naked polynucleotide injection, an effective dosage amount of DNA or
RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body
weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and
more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of
ordinary skill will appreciate, this dosage will vary according to the tissue site of
35 injection. The appropriate and effective dosage of nucleic acid sequence can readily be
determined by those of ordinary skill in the art and may depend on the condition being
treated and the route of administration. The preferred route of administration is by the

parenteral route of injection into the interstitial space of tissues. However, other
5 parenteral routes may also be used, such as, inhalation of an aerosol formulation
particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the
nose. In addition, naked polynucleotide constructs can be delivered to arteries during
angioplasty by the catheter used in the procedure.

10 The dose response effects of injected polynucleotide in muscle in vivo is
determined as follows. Suitable template DNA for production of mRNA coding for
polypeptide of the present invention is prepared in accordance with a standard
recombinant DNA methodology. The template DNA, which may be either circular or
linear, is either used as naked DNA or complexed with liposomes. The quadriceps
15 muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by
intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the
anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is
injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute,
20 approximately 0.5 cm from the distal insertion site of the muscle into the knee and about
0.2 cm deep. A suture is placed over the injection site for future localization, and the skin
is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared
25 by excising the entire quadriceps. Every fifth 15 um cross-section of the individual
quadriceps muscles is histochemically stained for protein expression. A time course for
protein expression may be done in a similar fashion except that quadriceps from different
mice are harvested at different times. Persistence of DNA in muscle following injection
may be determined by Southern blot analysis after preparing total cellular DNA and
30 HIRT supernatants from injected and control mice. The results of the above
experimentation in mice can be used to extrapolate proper dosages and other treatment
parameters in humans and other animals using naked DNA.

35 **Example 38 - Transgenic Animals.**

The polypeptides of the invention can also be expressed in transgenic animals.
Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea
pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons,

monkeys, and chimpanzees may be used to generate transgenic animals. In a specific
5 embodiment, techniques described herein or otherwise known in the art, are used to
express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e.,
polynucleotides of the invention) into animals to produce the founder lines of transgenic
10 animals. Such techniques include, but are not limited to, pronuclear microinjection
(Paterson et al., *Appl. Microbiol. Biotechnol.* 40:691-698 (1994); Carver et al.,
Biotechnology (NY) 11:1263-1270 (1993); Wright et al., *Biotechnology (NY)* 9:830-834
(1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene
transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148-6152
15 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al.,
Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, *Mol Cell. Biol.*
3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene
gun (see, e.g., Ulmer et al., *Science* 259:1745 (1993); introducing nucleic acid constructs
into embryonic pluripotent stem cells and transferring the stem cells back into the
20 blastocyst; and sperm-mediated gene transfer (Lavitrano et al., *Cell* 57:717-723 (1989);
etc. For a review of such techniques, see Gordon, "Transgenic Animals" *Intl. Rev. Cytol.*
115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones
25 containing polynucleotides of the invention, for example, nuclear transfer into enucleated
oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence
(Campell et al., *Nature* 380:64-66 (1996); Wilmut et al., *Nature* 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in
all their cells, as well as animals which carry the transgene in some, but not all their cells,
30 i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene
or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail
tandems. The transgene may also be selectively introduced into and activated in a
particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al.,
Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for
35 such a cell-type specific activation will depend upon the particular cell type of interest,
and will be apparent to those of skill in the art. When it is desired that the polynucleotide
transgene be integrated into the chromosomal site of the endogenous gene, gene targeting

is preferred. Briefly, when such a technique is to be utilized, vectors containing some
5 nucleotide sequences homologous to the endogenous gene are designed for the purpose
of integrating, via homologous recombination with chromosomal sequences, into and
disrupting the function of the nucleotide sequence of the endogenous gene. The transgene
may also be selectively introduced into a particular cell type, thus inactivating the
10 endogenous gene in only that cell type, by following, for example, the teaching of Gu et
al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such
a cell-type specific inactivation will depend upon the particular cell type of interest, and
will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant
15 gene may be assayed utilizing standard techniques. Initial screening may be accomplished
by Southern blot analysis or PCR techniques to analyze animal tissues to verify that
integration of the transgene has taken place. The level of mRNA expression of the
transgene in the tissues of the transgenic animals may also be assessed using techniques
which include, but are not limited to, Northern blot analysis of tissue samples obtained
20 from the animal, in situ hybridization analysis, and reverse transcriptase-PCR(RT-PCR)..
Samples of transgenic gene-expressing tissue may also be evaluated
immunocytochemically or immunohistochemically using antibodies specific for the
transgene product.

25 Once the founder animals are produced, they may be bred, inbred, outbred, or
crossbred to produce colonies of the particular animal. Examples of such breeding
strategies include, but are not limited to: outbreeding of founder animals with more than
one integration site in order to establish separate lines; inbreeding of separate lines in
order to produce compound transgenics that express the transgene at higher levels
30 because of the effects of additive expression of each transgene; crossing of heterozygous
transgenic animals to produce animals homozygous for a given integration site in order
to both augment expression and eliminate the need for screening of animals by DNA
analysis; crossing of separate homozygous lines to produce compound heterozygous or
homozygous lines; and breeding to place the transgene on a distinct background that is
35 appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited
to, animal model systems useful in elaborating the biological function of polypeptides of

the present invention, studying diseases, disorders, and/or conditions associated with
5 aberrant expression, and in screening for compounds effective in ameliorating such
diseases, disorders, and/or conditions.

Example 39 - Knock-Out Animals.

10 Endogenous gene expression can also be reduced by inactivating or "knocking
out" the gene and/or its promoter using targeted homologous recombination. (E.g., see
Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512
(1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by
reference herein in its entirety). For example, a mutant, non-functional polynucleotide of
15 the invention (or a completely unrelated DNA sequence) flanked by DNA homologous
to the endogenous polynucleotide sequence (either the coding regions or regulatory
regions of the gene) can be used, with or without a selectable marker and/or a negative
selectable marker, to transfect cells that express polypeptides of the invention in vivo. In
another embodiment, techniques known in the art are used to generate knockouts in cells
20 that contain, but do not express the gene of interest. Insertion of the DNA construct, via
targeted homologous recombination, results in inactivation of the targeted gene. Such
approaches are particularly suited in research and agricultural fields where modifications
to embryonic stem cells can be used to generate animal offspring with an inactive
targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, supra). However
25 this approach can be routinely adapted for use in humans provided the recombinant DNA
constructs are directly administered or targeted to the required site in vivo using
appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to
30 express the polypeptides of the invention, or alternatively, that are genetically engineered
not to express the polypeptides of the invention (e.g., knockouts) are administered to a
patient in vivo. Such cells may be obtained from the patient (i.e., animal, including
human) or an MHC compatible donor and can include, but are not limited to fibroblasts,
bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial
35 cells etc. The cells are genetically engineered in vitro using recombinant DNA techniques
to introduce the coding sequence of polypeptides of the invention into the cells, or
alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence

associated with the polypeptides of the invention, e.g., by transduction (using viral
5 vectors, and preferably vectors that integrate the transgene into the cell genome) or
transfection procedures, including, but not limited to, the use of plasmids, cosmids,
YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the
polypeptides of the invention can be placed under the control of a strong constitutive or
10 inducible promoter or promoter/enhancer to achieve expression, and preferably secretion,
of the polypeptides of the invention. The engineered cells which express and preferably
secrete the polypeptides of the invention can be introduced into the patient systemically,
e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the
15 body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft;
genetically engineered endothelial cells can be implanted as part of a lymphatic or
vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and
Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by
reference herein in its entirety).

20 When the cells to be administered are non-autologous or non-MHC compatible
cells, they can be administered using well known techniques which prevent the
development of a host immune response against the introduced cells. For example, the
cells may be introduced in an encapsulated form which, while allowing for an exchange
25 of components with the immediate extracellular environment, does not allow the
introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include,
but are not limited to, animal model systems useful in elaborating the biological function
of polypeptides of the present invention, studying diseases, disorders, and/or conditions
30 associated with aberrant expression, and in screening for compounds effective in
ameliorating such diseases, disorders, and/or conditions.

Example 40 - Production Of An Antibody.

a) Hybridoma Technology

35 The antibodies of the present invention can be prepared by a variety of methods.
(See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing
HGPRBMY1 or HGPRBMY2 are administered to an animal to induce the production of

sera containing polyclonal antibodies. In a preferred method, a preparation of
5 HGPRBMY1 or HGPRBMY2 protein is prepared and purified to render it substantially
free of natural contaminants. Such a preparation is then introduced into an animal in
order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for protein HGPRBMY1 or HGPRBMY2 are
10 prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et
al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976);
Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y.,
pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with
HGPRBMY1 or HGPRBMY2 polypeptide or, more preferably, with a secreted
15 HGPRBMY1 or HGPRBMY2 polypeptide-expressing cell. Such polypeptide-expressing
cells are cultured in any suitable tissue culture medium, preferably in Earle's modified
Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C),
and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of
penicillin, and about 100 µg/ml of streptomycin.

20 The splenocytes of such mice are extracted and fused with a suitable myeloma
cell line. Any suitable myeloma cell line may be employed in accordance with the present
invention; however, it is preferable to employ the parent myeloma cell line (SP2O),
available from the ATCC. After fusion, the resulting hybridoma cells are selectively
25 maintained in HAT medium, and then cloned by limiting dilution as described by Wands
et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such
a selection are then assayed to identify clones which secrete antibodies capable of binding
the HGPRBMY1 or HGPRBMY2 polypeptide.

Alternatively, additional antibodies capable of binding to HGPRBMY1 or
30 HGPRBMY2 polypeptide can be produced in a two-step procedure using anti-idiotypic
antibodies. Such a method makes use of the fact that antibodies are themselves antigens,
and therefore, it is possible to obtain an antibody that binds to a second antibody. In
accordance with this method, protein specific antibodies are used to immunize an animal,
preferably a mouse. The splenocytes of such an animal are then used to produce
35 hybridoma cells, and the hybridoma cells are screened to identify clones which produce
an antibody whose ability to bind to the HGPRBMY1 or HGPRBMY2 protein-specific
antibody can be blocked by HGPRBMY1 or HGPRBMY2. Such antibodies comprise

anti-idiotypic antibodies to the HGPRBMY1 or HGPRBMY2 protein-specific antibody
5 and are used to immunize an animal to induce formation of further HGPRBMY1 or
HGPRBMY2 protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such
antibodies can be produced using genetic constructs derived from hybridoma cells
producing the monoclonal antibodies described above. Methods for producing chimeric
10 and humanized antibodies are known in the art and are discussed herein. (See, for review,
Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et
al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494;
Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature
15 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed

Against HGPRBMY1 or HGPRBMY2 From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a
library of antibody fragments which contain reactivities against HGPRBMY1 or
20 HGPRBMY2 to which the donor may or may not have been exposed (see e.g., U.S.
Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human
PBLs as described in PCT publication WO 92/01047. To rescue phage displaying
antibody fragments, approximately 109 E. coli harboring the phagemid are used to
25 inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-
AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to
inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10⁸ TU of delta gene 3 helper (M13 delta gene
III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for
30 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture
is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY
containing 100 µg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage
are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does
35 not encode gene III protein, hence the phage(mid) displaying antibody fragments have a
greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by
growing the helper phage in cells harboring a pUC19 derivative supplying the wild type

gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37°
5 C without shaking and then for a further hour at 37°C with shaking. Cells are spun down
(IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100
µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight,
shaking at 37°C. Phage particles are purified and concentrated from the culture medium
10 by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed
through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of
approximately 10¹³ transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immuntubes (Nunc) are coated overnight in PBS with
4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are
15 blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS.
Approximately 10¹³ TU of phage is applied to the tube and incubated for 30 minutes at
room temperature tumbling on an over and under turntable and then left to stand for
another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times
with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15
20 minutes on an under and over turntable after which the solution is immediately
neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml
of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C.
The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml
25 ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage
as described above to prepare phage for a subsequent round of selection. This process is
then repeated for a total of 4 rounds of affinity purification with tube-washing increased
to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of
30 selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al.,
1991) from single colonies for assay. ELISAs are performed with microtitre plates coated
with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate
pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see,
e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive
35 clones may also be further characterized by techniques known in the art, such as, for
example, epitope mapping, binding affinity, receptor signal transduction, ability to block
or competitively inhibit antibody/antigen binding, and competitive agonistic or

antagonistic activity.

5

Example 41 - Assays Detecting Stimulation Or Inhibition Of B Cell Proliferation And Differentiation.

10 Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations. One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

25 In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed Staphylococcus aureus Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of

CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed
5 by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well
plate to which are added 10⁵ B-cells suspended in culture medium (RPMI 1640
containing 10% FBS, 5 X 10⁻⁵M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and
10⁻⁵ dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated
10 by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor
addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only,
or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive
15 this treatment for 4 consecutive days, at which time they are sacrificed and various tissues
and serum collected for analyses. Comparison of H&E sections from normal spleens and
spleens treated with polypeptides of the invention identify the results of the activity of the
polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths,
and/or significant increases in the nucleated cellularity of the red pulp regions, which may
20 indicate the activation of the differentiation and proliferation of B-cell populations.
Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to
determine whether any physiological changes to splenic cells, such as splenic
disorganization, are due to increased B-cell representation within loosely defined B-cell
25 zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is
used to indicate whether the polypeptide specifically increases the proportion of ThB+,
CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in
30 vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are
compared between buffer and polypeptide-treated mice.

One skilled in the art could easily modify the exemplified studies to test the
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or
antagonists of polynucleotides or polypeptides of the invention.
35

Example 42 - T Cell Proliferation Assay.

A CD3-induced proliferation assay is performed on PBMCs and is measured by

the uptake of 3H-thymidine. The assay is performed as follows. Ninety-six well plates
5 are coated with 100 (l/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched
control mAb (B33.1) overnight at 4 degrees C (1 (g/ml in .05M bicarbonate buffer, pH
9.5), then washed three times with PBS. PBMC are isolated by F/H gradient
centrifugation from human peripheral blood and added to quadruplicate wells (5 x
10 104/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence
of varying concentrations of polypeptides of the invention (total volume 200 ul). Relevant
protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates
are spun for 2 min. at 1000 rpm and 100 (l of supernatant is removed and stored -20
degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is
15 observed. Wells are supplemented with 100 ul of medium containing 0.5 uCi of 3H-
thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and
incorporation of 3H-thymidine used as a measure of proliferation. Anti-CD3 alone is the
positive control for proliferation. IL-2 (100 U/ml) is also used as a control which
enhances proliferation. Control antibody which does not induce proliferation of T cells
20 is used as the negative controls for the effects of polypeptides of the invention.

One skilled in the art could easily modify the exemplified studies to test the
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or
antagonists of polynucleotides or polypeptides of the invention.

25 **Example 43 - Effect Of Polypeptides Of The Invention On The Expression Of MHC
Class II, Costimulatory And Adhesion Molecules And Cell Differentiation Of
Monocytes And Monocyte-Derived Human Dendritic Cells.**

Dendritic cells are generated by the expansion of proliferating precursors found
30 in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for
7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the
characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and
MHC class II antigens). Treatment with activating factors, such as TNF-, causes a rapid
change in surface phenotype (increased expression of MHC class I and II, costimulatory
35 and adhesion molecules, downregulation of FC(RII, upregulation of CD83). These
changes correlate with increased antigen-presenting capacity and with functional
maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3
5 days with increasing concentrations of polypeptides of the invention or LPS (positive
control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then
incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies
for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by
10 flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in
particular IL-12, are important in the initiation of T-cell dependent immune responses.
IL-12 strongly influences the development of Th1 helper T-cell immune response, and
induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release
15 as follows. Dendritic cells (10⁶/ml) are treated with increasing concentrations of
polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture
as positive control. Supernatants from the cell cultures are then collected and analyzed
for IL-12 content using commercial ELISA kit(e.g., R & D Systems (Minneapolis, MN)).
The standard protocols provided with the kits are used.

20 Effect on the expression of MHC Class II, costimulatory and adhesion molecules.
Three major families of cell surface antigens can be identified on monocytes: adhesion
molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of
the expression of MHC class II antigens and other costimulatory molecules, such as B7
25 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and
ability to induce T cell activation. Increase expression of Fc receptors may correlate with
improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are
treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS
30 (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and
then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal
antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are
analyzed by flow cytometry on a FACScan (Becton Dickinson).

35 Monocyte activation and/or increased survival. Assays for molecules that activate
(or alternatively, inactivate) monocytes and/or increase monocyte survival (or
alternatively, decrease monocyte survival) are known in the art and may routinely be
applied to determine whether a molecule of the invention functions as an inhibitor or

activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be
5 screened using the three assays described below. For each of these assays, Peripheral
blood mononuclear cells (PBMC) are purified from single donor leukopacks (American
Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma).
Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

10 Monocyte Survival Assay. Human peripheral blood monocytes progressively lose
viability when cultured in absence of serum or other stimuli. Their death results from
internally regulated process (apoptosis). Addition to the culture of activating factors, such
as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation.
Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are
15 cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in
the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying
concentrations of the compound to be tested. Cells are suspended at a concentration of
2 x 10⁶/ml in PBS containing PI at a final concentration of 5 (g/ml, and then incubated
at room temperature for 5 minutes before FACScan analysis. PI uptake has been
20 demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is
their regulatory activity on other cellular populations of the immune system through the
release of cytokines after stimulation. An ELISA to measure cytokine release is
25 performed as follows. Human monocytes are incubated at a density of 5x10⁵ cells/ml
with increasing concentrations of the a polypeptide of the invention and under the same
conditions, but in the absence of the polypeptide. For IL-12 production, the cells are
primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS
(10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until
30 use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a
commercially available ELISA kit(e.g., R & D Systems (Minneapolis, MN)) and applying
the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at 2-1x10⁵ cell/well.
Increasing concentrations of polypeptides of the invention are added to the wells in a total
35 volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics).
After 3 days incubation, the plates are centrifuged and the medium is removed from the
wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM

NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red
5 and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates
are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH
per well. The absorbance is read at 610 nm. To calculate the amount of H₂O₂ produced
by the macrophages, a standard curve of a H₂O₂ solution of known molarity is performed
10 for each experiment.

One skilled in the art could easily modify the exemplified studies to test the
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or
antagonists of polynucleotides or polypeptides of the invention.

15 **Example 44 - Biological Effects of HGPRBMY2 Polypeptides of the Invention.**

Astrocyte and Neuronal Assays.

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and
purified as described above, can be tested for activity in promoting the survival, neurite
outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the
20 proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The
selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1
and FGF-2 in cortical structures and on the previously reported enhancement of cortical
neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for
example, can be used to elucidate a polypeptide of the invention's activity on these cells.
25

Moreover, previous reports describing the biological effects of FGF-2 (basic
FGF) on cortical or hippocampal neurons in vitro have demonstrated increases in both
neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor
promotes survival of dissociated hippocampal neurons and enhances neurite extension."
30 *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference
in its entirety). However, reports from experiments done on PC-12 cells suggest that these
two responses are not necessarily synonymous and may depend on not only which FGF
is being tested but also on which receptor(s) are expressed on the target cells. Using the
primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention
35 to induce neurite outgrowth can be compared to the response achieved with FGF-2 using,
for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays.

5 Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated
10 for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a
15 CytoFluor fluorescence reader. For the PGE2 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1(for 24 hours. The supernatants are collected and assayed for PGE2 by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung
20 fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1(for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

25 Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

30 Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-
35 tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP+) and released. Subsequently, MPP+ is actively accumulated in dopaminergic neurons by the high-

5 affinity reuptake transporter for dopamine. MPP⁺ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

10 It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

15 Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival in vitro and it can also be tested in vivo for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined in vitro in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm² on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days in vitro and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

30 Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving in vitro. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

35 One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or

antagonists of polynucleotides or polypeptides of the invention.

5

Example 45 - Stimulation Of Nitric Oxide Production By Endothelial Cells.

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

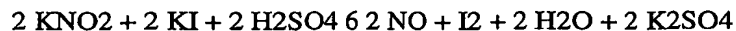
10

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

15

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:

20



The standard calibration curve is obtained by adding graded concentrations of KNO₂ (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and H₂SO₄. The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per 1x10⁶ endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak et al. Biochem. and Biophys. Res. Comm. 217:96-105 (1995).

25
30
35

One skilled in the art could easily modify the exemplified studies to test the

activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or
5 antagonists of polynucleotides or polypeptides of the invention.

Example 46 - Effect Of Polypeptides Of The Invention On Vasodilation.

Since dilation of vascular endothelium is important in reducing blood pressure,
the ability of polypeptides of the invention to affect the blood pressure in spontaneously
10 hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900
mg/kg) of the polypeptides of the invention are administered to 13-14 week old
spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM.
Statistical analysis are performed with a paired t-test and statistical significance is defined
15 as $p < 0.05$ vs. the response to buffer alone.

One skilled in the art could easily modify the exemplified studies to test the
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or
antagonists of polynucleotides or polypeptides of the invention.

20 **Example 47 - Peripheral Arterial Disease Model.**

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic
strategy to obtain restoration of blood flow around the ischemia in case of peripheral
arterial diseases. The experimental protocol includes:

- 25 a) One side of the femoral artery is ligated to create ischemic muscle of
the hindlimb, the other side of hindlimb serves as a control.
- b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is
delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3
weeks.
- 30 c) The ischemic muscle tissue is collected after ligation of the femoral
artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention
and histology. Biopsy is also performed on the other side of normal muscle of the
contralateral hindlimb.

One skilled in the art could easily modify the exemplified studies to test the
35 activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or
antagonists of polynucleotides or polypeptides of the invention.

Example 48 - Ischemic Myocardial Disease Model.

5 A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

10 a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

15 c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

20

EQUIVALENTS AND REFERENCES

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such equivalents are intended to be within the scope of the following claims.

25 All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

35

What is claimed is:

- 5 1. An isolated nucleic acid molecule consisting of a polynucleotide having
a nucleotide sequence selected from the group consisting of:
- (a) a polynucleotide encoding a polypeptide of SEQ ID NO:2;
 (b) an isolated polynucleotide consisting of nucleotides 250 to 1323 of
10 SEQ ID NO:1, wherein said nucleotides encode a polypeptide corresponding to amino
acids 2 to 359 of SEQ ID NO:2 minus the start codon;
 (c) an isolated polynucleotide consisting of nucleotides 247 to 1323 of
SEQ ID NO:1, wherein said nucleotides encode a polypeptide corresponding to amino
acids 1 to 329 of SEQ ID NO:2 including the start codon;
15 (d) a polynucleotide encoding the HGPRBMY1 polypeptide encoded
by the cDNA clone contained in ATCC Deposit No. XXXXXX;
 (e) a polynucleotide which represents the complimentary sequence
(antisense) of SEQ ID NO:1;
 (a) a polynucleotide encoding a polypeptide of SEQ ID NO:14;
20 (b) an isolated polynucleotide consisting of nucleotides 362 to 1651 of
SEQ ID NO:13, wherein said nucleotides encode a polypeptide corresponding to amino
acids 2 to 431 of SEQ ID NO:14 minus the start codon;
 (c) an isolated polynucleotide consisting of nucleotides 359 to 1651 of
25 SEQ ID NO:13, wherein said nucleotides encode a polypeptide corresponding to
amino acids 1 to 431 of SEQ ID NO:13 including the start codon;
 (d) a polynucleotide encoding the HGPRBMY2 polypeptide encoded
by the cDNA clone contained in ATCC Deposit No. XXXXXX; and
 (e) a polynucleotide which represents the complimentary sequence
30 (antisense) of SEQ ID NO:13.
2. The isolated nucleic acid molecule of claim 1, wherein the
polynucleotide comprises a nucleotide sequence encoding a human G-
protein coupled receptor protein.
- 35 3. A recombinant vector comprising the isolated nucleic acid molecule of
claim 2.
4. A recombinant host cell comprising the recombinant vector of claim 3.

- 5 5. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of:
- (a) a full length protein of SEQ ID NO:2;
- (b) a polypeptide corresponding to amino acids 2 to 359 of SEQ ID NO:2, wherein said amino acids 2 to 359 comprise a polypeptide of SEQ ID NO:2 minus the start methionine;
- 10 (c) a polypeptide corresponding to amino acids 1 to 359 of SEQ ID NO:2;
- (d) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXXX;
- (e) a full length protein of SEQ ID NO:14;
- 15 (f) a polypeptide corresponding to amino acids 2 to 431 of SEQ ID NO:14, wherein said amino acids 2 to 431 comprise a polypeptide of SEQ ID NO:14 minus the start methionine;
- (g) a polypeptide corresponding to amino acids 1 to 431 of SEQ ID NO:14; and
- 20 (h) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXXX.
6. A cell comprising the polypeptide of claim 4 and a member selected from the group consisting of NFAT/CRE, and NFAT G alpha 15.
- 25 7. A method of screening for candidate compounds capable of modulating activity of a G-protein coupled receptor-encoding polypeptide, comprising:
- (a) contacting a test compound with the cell according to claim 6; and
- (b) selecting as candidate modulating compounds those test compounds that
- 30 modulate activity of the G-protein coupled receptor polypeptide.
8. A polypeptide that binds to the polypeptide of claim 1 selected from the group consisting of SEQ ID NO:32, 33, 34, and 35.
- 35 9. The polypeptide of claim 8 wherein said polypeptide modulates the G-protein coupled receptor of claim 5.

10. A method of screening for candidate compounds capable of modulating
5 activity of a G-protein coupled receptor-encoding polypeptide,
comprising:
- (a). contacting the modulating polypeptide of claim 9 with the cell
according to claim 4;
 - (b). contacting a test compound with said cell; and
 - 10 (c). selecting as candidate modulating compounds those test
compounds that modulate activity of the G-protein coupled receptor polypeptide.
11. A method of modulating the G-protein coupled receptor activity of the
polypeptide of claim 5 comprising an effective amount of the polypeptide
15 of claim 9.
12. The method for the treatment of immune-related disorders comprising a
member of the group consisting of:
- (a) administering to the subject a therapeutically effective
amount of a HGPRBMY1 polypeptide;
 - 20 (b) modulating the activity of a HGPRBMY1 polypeptide;
 - (c) administering to the subject a therapeutically effective
amount of a HGPRBMY1 polypeptide wherein the
HGPRBMY1 polypeptide is contained in a
pharmaceutical composition;
 - 25 (d) modulating the activity of a HGPRBMY1 polypeptide
wherein the HGPRBMY1 polypeptide is HGPRBMY1 or
a functionally equivalent derivative thereof;
 - (e) wherein the HGPRBMY1 polypeptide is HGPRBMY1 or
30 a functionally equivalent derivative thereof wherein the
HGPRBMY1 polypeptide is HGPRBMY1 or a
functionally equivalent derivative thereof wherein the
method comprises administering an effective amount of a
compound that agonizes or antagonizes the activity of the
HGPRBMY1 polypeptide;
 - 35 (f) administering an effective amount of a compound that
decreases expression of a HGPRBMY1 gene;

- 5 (g) administering an effective amount of a compound that decreases expression of a HGPRBMY1 gene in which the compound is an oligonucleotide encoding an antisense or ribozyme molecule that targets HGPRBMY1 transcripts and inhibits translation; and
- 10 (h) administering an effective amount of a compound that increases expression of a HGPRBMY1 gene.
13. The method for the treatment of heart-related disorders comprising a member of the group consisting of:
- 15 (i) administering to the subject a therapeutically effective amount of a HGPRBMY2 polypeptide;
- (j) modulating the activity of a HGPRBMY2 polypeptide;
- (k) administering to the subject a therapeutically effective amount of a HGPRBMY2 polypeptide wherein the
20 HGPRBMY2 polypeptide is contained in a pharmaceutical composition;
- (l) modulating the activity of a HGPRBMY2 polypeptide wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof;
- 25 (m) wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof wherein the
30 method comprises administering an effective amount of a compound that agonizes or antagonizes the activity of the HGPRBMY2 polypeptide;
- (n) administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene;
- 35 (o) administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene in which the compound is an oligonucleotide encoding an antisense or

- ribozyme molecule that targets HGPRBMY2 transcripts
and inhibits translation; and
- (p) administering an effective amount of a compound that
increases expression of a HGPRBMY2 gene.
14. A method for preventing, treating, or ameliorating a medical condition,
comprising the step of administering to a mammalian subject a
therapeutically effective amount of a member of the group consisting of:
- (a) a polynucleotide of claim 1;
 - (b) a polypeptide of claim 5;
 - (c) an antagonist of the polypeptide of claim 5;
 - (d) an agonist of the polypeptide of claim 5;
 - (e) an antagonist of the polynucleotide of claim 1;
 - (f) a agonist of the polynucleotide of claim 1;
 - (g) the modulatory polypeptide of claim 9; and
 - (h) an antibody directed against the polypeptide of claim 5.
15. The method of preventing, treating, or ameliorating a medical condition
of claim 14, wherein the medical condition is selected from the group
consisting of an immune disorder, a hematopoietic disorder, a pulmonary
disorder, and a gastrointestinal disorder.
16. The method of preventing, treating, or ameliorating a medical condition
of claim 14, wherein the medical condition is selected from the group
consisting of a cardiovascular disorder, a neural disorder, and a
reproductive disorder.
17. The method of preventing, treating, or ameliorating a medical condition
of claim 14, wherein the medical condition is a cell cycle defect, a
disorder related to aberrant phosphorylation, a disorder related to aberrant
signal transduction, a proliferating disorder, an autoimmune disorder, a
disorder related to hyper immune activity, an inflammatory condition, a
disorder related to aberrant acute phase responses, a hypercongenital
condition, a birth defect, a necrotic lesion, a wound, organ transplant
rejection, and a condition related to organ transplant rejection.

18. The method of preventing, treating, or ameliorating a medical condition
5 of claim 14, wherein the medical condition is a metabolic disorder,
obesity, and pain.
19. The method of preventing, treating, or ameliorating a medical condition
10 of claim 14, wherein the condition is a condition related to aberrant cell
cycle regulation, aberrant p27 regulation, aberrant apoptosis regulation,
aberrant I κ B regulation, aberrant NF κ B regulation, aberrant DNA repair,
and aberrant cellular phosphorylation.
20. The isolated polypeptide of claim 5, wherein the full length protein
15 comprises sequential amino acid deletions from either the C-terminus or
the N-terminus.
- 20
- 25
- 30
- 35

GGCCGCCCTTT	GCAAGGTTGC	TGGACAGATG	GAAGTGGAAAG	GGCAGCCGTC
TGCCGCCCAC	GAACACCTTC	TCAAGCACTT	TGAGTGACCA	CGGCTTGCAA
GCTGGTGGCT	GGCCCCCGA	GTCCCGGGCT	CTGAGGCACG	GCCGTCGACT
TAAGCGTTGC	ATCCTGTTAC	CTGGAGACCC	TCTGAGCTCT	CACCTGCTAC
TTCTGCCGCT	GCTTCTGCAC	AGAGCCCGGG	CGAGGACCCC	TCCAGG
ATGCAGGTCC	CGAACAGCAC	CGGCCCGGAC	AACGCGACGC	TGCAGATGCT
GCGGAACCCG	GCGATCGCGG	TGGCCCTGCC	CGTGGTGTAC	TCGCTGGTGG
CGGCGGTACG	CATCCCGGGC	AACCTCTTCT	CTCTGTGGGT	GCTGTGCCGG
CGCATGGGGC	CCAGATCCCC	GTCGGTCATC	TTCATGATCA	ACCTGAGCGT
CACGGACCTG	ATGCTGGCCA	GCGTGTTCGC	TTTCCAAATC	TACTACCATT
GCAACCGCCA	CCACTGGGTA	TTCGGGGTGC	TGCTTTGCAA	CGTGGTGACC
GTGGCCTTTT	ACGCAAACAT	GTATTCCAGC	ATCCTCACCA	TGACCTGTAT
CAGCGTGGAG	CGCTTCCTGG	GGGTCCGTGA	CCCGCTCAGC	TCCAAGCGCT
GGCGCCGCCG	TCGTTACGCG	GTGGCCGCGT	GTGCAGGGAC	CTGGCTGCTG
CTCCTGACCG	CCCTGTCCCC	GCTGGCGCGC	ACCGATCTCA	CCTACCCGGT
GCACGCCCTG	GGCATCATCA	CCTGCTTCGA	CGTCCTCAAG	TGGACGATGC
TCCCCAGCGT	GGCCATGTGG	GCCGTGTTCC	TCTTCACCAT	CTTCATCCTG
CTGTTCCCTA	TCCCGTTCGT	GATCACCCTG	GCTTGTTACA	CGGCCACCAT
CCTCAAGCTG	TTGCGCACGG	AGGAGGCGCA	CGGCCGGGAG	CAGCGGAGGC
GCGCGGTGGG	CCTGGCCGCG	GTGGTCTTGC	TGGCCTTTGT	CACCTGCTTC
GCCCCCAACA	ACTTCGTGCT	CCTGGCGCAC	ATCGTGAGCC	GCCTGTTCTA
CGGCAAGAGC	TACTACCACG	TGTACAAGCT	CACGCTGTGT	CTCAGCTGCC
TCAACAACCTG	TCTGGACCCG	TTTGTTTTAT	ACTTTGCGTC	CCGGGAATTC
CAGCTGCGCC	TGCGGGAATA	TTTGGGCTGC	CGCCGGGTGC	CCAGAGACAC
CCTGGACACG	CGCCGCGAGA	GCCTCTTCTC	CGCCAGGACC	ACGTCCGTGC
GCTCCGAGGC	CGGTGCGCAC	CCTGAAGGGA	TGGAGGGAGC	CACCAGGCCC
GGCCTCCAGA	GGCAGGAGAG	TGTGTTC		
TCCCTGCTGA	CATCGTCCCT	TAGTTGTGGT	TCTGGCCTTC	TCCATTCTCC
TCCAGGGGTT	CTGGTCTCCG	TAGCCCGGTG	CACGCCGAAA	TTTCTGTTTA
TTTCACTCAG	GGGCACTGTG	GTTGCTGTGG	TTGGAATTCT	TCTTTCAGAG
GAGCGCCTGG	GGCTCCTGCA	AGTCAGCTAC	TCTCCGTGCC	CACTTCCCCT
CACACACACA	CCCCCTCGT	GCCGAATTCT	T	

FIG. 1

MQVPNSTGPDNATLQMLRNPAIAVALPVVYSLVAAVSIPGNLFSLWVLCRRMGPRSPSVI
FMINLSVTDLMLASVLPFQIYYHCNRHHWVFGVLLCNVVTVAFYANMYSSILTMTCSVE
RFLGVLYPLSSKRWRRRRYAVAACAGTWLLLLTALSPLARTDLTYPVHALGIITCFDVLK
WTMLPSVAMWAVFLFTIFILLFLIPFVITVACYTATILKLLRTEEAHGREQRRRAVGLAA
VLLAFVTCFAPNNFVLLAHIVSRLFYGKSYHVYKLTCLCLNCLDPFVYFASREF
QLRLREYLGCRRVPRDTLDTRESLFSARTTSVRSEAGAHPEGMEGATRPGLQRQESVF

FIG. 2

```
1      MQVPNSTGPD NATLQMLRNP AIAVALPVVY SLVAAVSIPG NLFSLWVLCR
51     RMGPRSPSVI FMINLSVTDL MLASVLPFQI YYHCNRHHWV FGVLLCNVVT
101    VAFYANMYSS ILTMTCSIVE RFLGVLYPLS SKRWRRRRYA VAACAGTWLL
151    LLTALSPLAR TDLTYPVHAL GIITCFDVLK WTMLPSVAMW AVFLFTIFIL
201    LFLIPFVITV ACYTATILKL LRTEEAHGRE QRRRAVGLAA VVLLAFVTCF
251    APNNFVLLAH IVSRLFYGKS YYHVYKLTLC LSCLNNCLDP FVYYFASREF
301    QLRLREYLG C RRVPRDTLDT RRESLFSART TSVRSEAGAH PEGMEGATRP
351    GLQRQESVF
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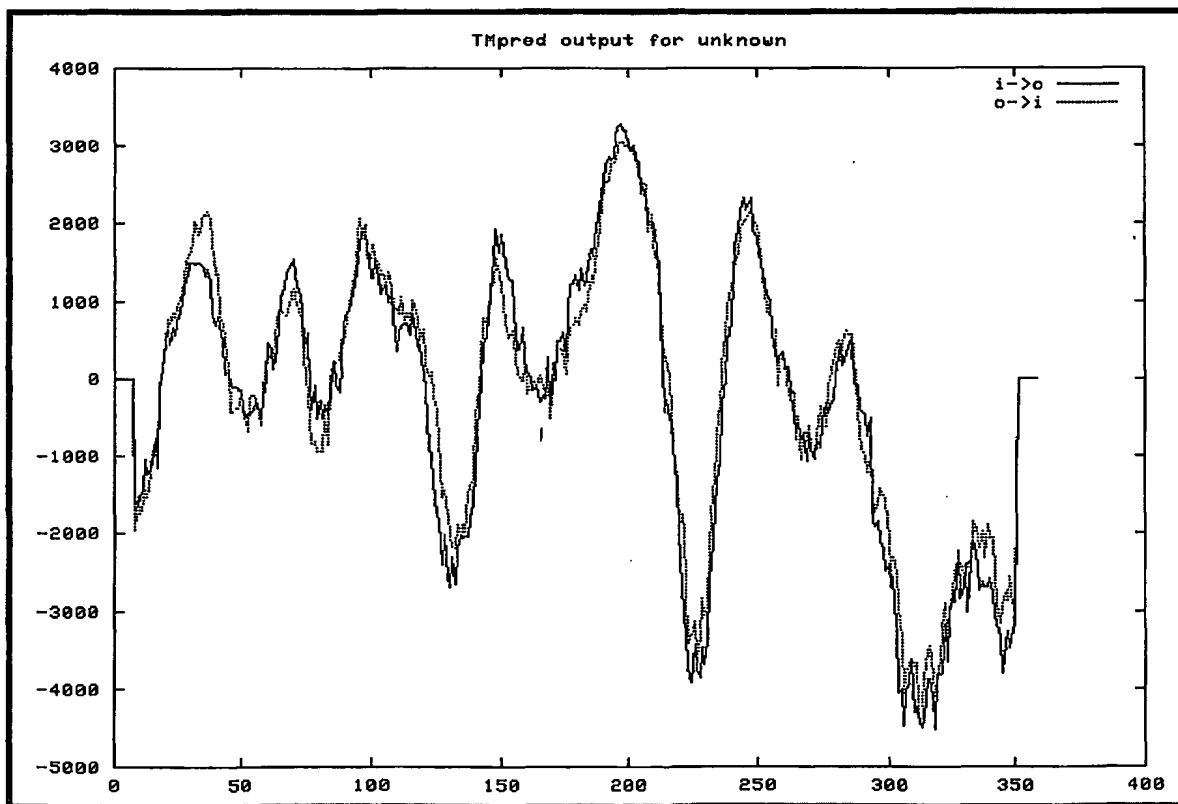


FIG. 3

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par2_human      ~~~~~MRSPSAAWLLGMA.ILLAASLSC.SGTIQGTNRSSKGRSLIGKVD..GT.....
par3_human      ~~~~~MKALIFAAAGLLLLLLPTFCQSGMENDTNNAKPTIPIKTFR..GAPPNSF
thrombin_Xeno    MMELRVLLLLLLLLLLTLLGAMGSLC..LANSDTQAKGAHS..NNMTT..KTPRIFDDSESEF
thrombin_human   ~~~~~MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATDPRSF..LLRNPNDKY
HGPRBMY1        ~~~~~
par4_human      ~~~~~MWGRLLWPLVLGFSLSGGTQT
p2y9_human      ~~~~~

par2_human      .....SHVT.G.KGVTVETVFSVDEFSAS.....VLTGKLTIV
par3_human      EEFPPSALE.GWTGATITVKIKCPEESASHLH.V.....K..NATMG...YLTSSLSTK
thrombin_Xeno    EEIPWDELDESGECSGDQAFVSRSAKPIRRN.....ITKE...AE.Q...YLSQWLTK
thrombin_human   EPF.WEDEKNESGLTEYRLVSINKSSPLOKQ.LPAFISED...AS.G...YLTSSWLTL
HGPRBMY1        MQ.VPNSTGPD..NATLQ...MERNPAIAV
par4_human      PSVYDESGSTGGGDDSTPSITPAPRGYPGQVC.ANDSDTLELPDSSRA...LILGWVPTR
p2y9_human      ~~~~~MGDRRFIDFQFQDSNSSLRPLGNATANNTCTVDDSFKN

par2_human      FLPIVYTVFVVGLPSSNGMALWVFLFRITKKEHFAVITYMANLALADLLSVIWEPLKIAYHI
par3_human      LIPATYLVFVVGVPANAVTLWMLFFERTSICTVFEY.TNLATADELFCVTLPPFKIAYHI
thrombin_Xeno    FVPSLYTVVFEIVGLPLNLLAKITIFLRMRVKEKPAVVYMLNLATADVFVSVLPFKIAYHI
thrombin_human   FVPSVYTGFFVVSPLNIMATVVFILKMKVKKPAVVYMLNLATADVLFVSVLPFKISYYF
HGPRBMY1        ALPVVYSIVAAVSHPEGNLFSLWVLCRRMGPPSESVIFMNLSTVDLMLASVLPFQIYYHC
par4_human      LVPALVGLVVLVGLPANGFALWVLATQA.PRLSTMLLNLATADLLLALALPFIAYHI
p2y9_human      LAGAVYSVVEILGLITNSVSLFVFCFRMKMRSETAIFITNLAVSDLLFVCTLPKITYNF

par2_human      HGNNWVYGEALCNVLIGFYGNMYCSILFMTCLSVORYWVTVNPMGH.SRKKNATIGIS
par3_human      NGNNWVYGEVLCRATTVIFYGNMYCSILLACISINRYLATVHPFTYRGLPKHTYALVTC
thrombin_Xeno    SGNDWLCGPGMCRTVTAIFYGNMYCSVLLIASISVDRFLAVVYPMHSLSWTMSRAYMAC
thrombin_human   SGSDWQFCSELCREFTAFIFYGNMYASILLMTVISIDRFLAVVYPMOSLSWTGLGRASFTC
HGPRBMY1        NRHHWVFGVLLCNVTVAFYANMYSSILMTTCISVERFLGVLYPLSSKRWRERRRYAAAC
par4_human      RGRWPFCGAACRLATAALYGHMYGSVLLAAMSEDRYLALVHPIRARALGRRLALGLC
p2y9_human      N.RHWPFCITLCKISGTALTNLYGSMFLTCISVDRFLAVVYFPRSRFTITRNSAIVC

par2_human      LAGWLLTLLVTIPLYVVKOTIFI PALNITTCHDV..LPEQLLVGDMFNY.FLSLA..GVF
par3_human      GLANATVFLYMLPFFTLKQEYDVQPDITTCDDVHNTCESSPFQLY.Y.FISLA.FFGF
thrombin_Xeno    SFHWLTSASTIPLLYTEOTQKIPRLDITTCDDVLDLKDLD...FYIYFSSFC..LIF
thrombin_human   LAIWALAGVVPVVKETIQVGLNITTCHDVNETLEG...YVAYVSAFS.AVFE
HGPRBMY1        AGTWLLITALSPLARTDITYPVHALGITCEDVLKWTMLPSVAMWAVLELIE..IELF
par4_human      MAAWLMAAALAPLITLQRTFRLARS DRVLC HDALP...LDAQASHWQPAFCLA..LIGC
p2y9_human      AGVWILVLSGGISASLFSTI.NVNNAT.TTCFEGFS...KRVWKTYLSKITLIEVVGF

par2_human      LFPAPLTASAYV.LMIRMRSSAMDENSEKRRKRAIKLIVTVIAMYLCIFPSNLLLVH
par3_human      LIPFVLITYCYA.AIIRTL..NAYDH...RWLWYVKASLILVITICFAPSNIILITH
thrombin_Xeno    FVPFLITTCYIG.IIRSLSSSSIENSCKIT..RALFLAVVLCVFIIICEGPTNVLELTH
thrombin_human   FVPLIISTVCYVS.IIRCLSSSAVANRSKKS..RALFLSAAVFCIFIIICEGPTNVLLIAH
HGPRBMY1        LIPFVITVACYTATILKLRTTEEAGREQR..RAVGLAAVLLAFVTCFAPNNEVLLAH
par4_human      FLPLLAMLLCY.GATHTLAASG.....RRYGHALRLTAVVLASAVAFVPSNLLLLH
p2y9_human      LIPLITLWVSC.SSVVLTIRKPA TL SQIGTNKKV LKMITVHMAFVVCFEYNSVLFY

```

FIG. 4

```

par2_human      YFLI.KSQGQ...SHMYALYVALCLSLNSCLDPFVYYEVSHDERDHAKNALCRSVR
par3_human      HANY.YYNNT...DGLYFYIYLIALCLGSLNSCLDPFLYFLMSKT.RNHS.TAYLTK~~~
thrombin_Xeno   V..LQEAN.....EFLYFAYILSACVGSVSCCLDPFLIYYVASSQCORYIYSLCCRKVS
thrombin_human  YSFLSHTSTT...EAAVFAYLLCVQVSSISSCCLDPFLIYYVASSQCORYMYSILCKESS
HGPRBMY1        ..IVSRIFYG....KSYVHYVLLTLCLSLNCLDPFVYYEASREFOLRREYLGCRRV.
par4_human      YSDPSPSAWG...N.LYGAVVPSLALSLNSCLDPFIYYIVSASFRDKVRAGLFQSPG
p2y9_human      ALVRSQAITNCFLERFAKIVPLTLCLAILNCCFDPFIYYETLESFCKSFYINAHIRMES

par2_human      TVKQMQVSLTSKKHSRQSSSYSSSSTTVKTSY~~~~~
par3_human      ~~~~~~
thrombin_Xeno   EPGS..STGQLMSTAMKNDNCSTNAKSSIYKKLTA~~~~~
thrombin_human  DPSSYNSSGQLM..ASKMDTCSSNLNNSIYKKLIT~~~~~
HGPRBMY1        .PRDTLDIRRESLFSAR..TTSVRSEAGAHPEGMEGATRPGLRQESVF
par4_human      DTVASKASAEGGSRGMGTHSSLLQ~~~~~
p2y9_human      LFKTETPLTTKPSLPAIQEEVSDQTTNNGGELMTESTF~~~~~

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FIG. 4 Continued

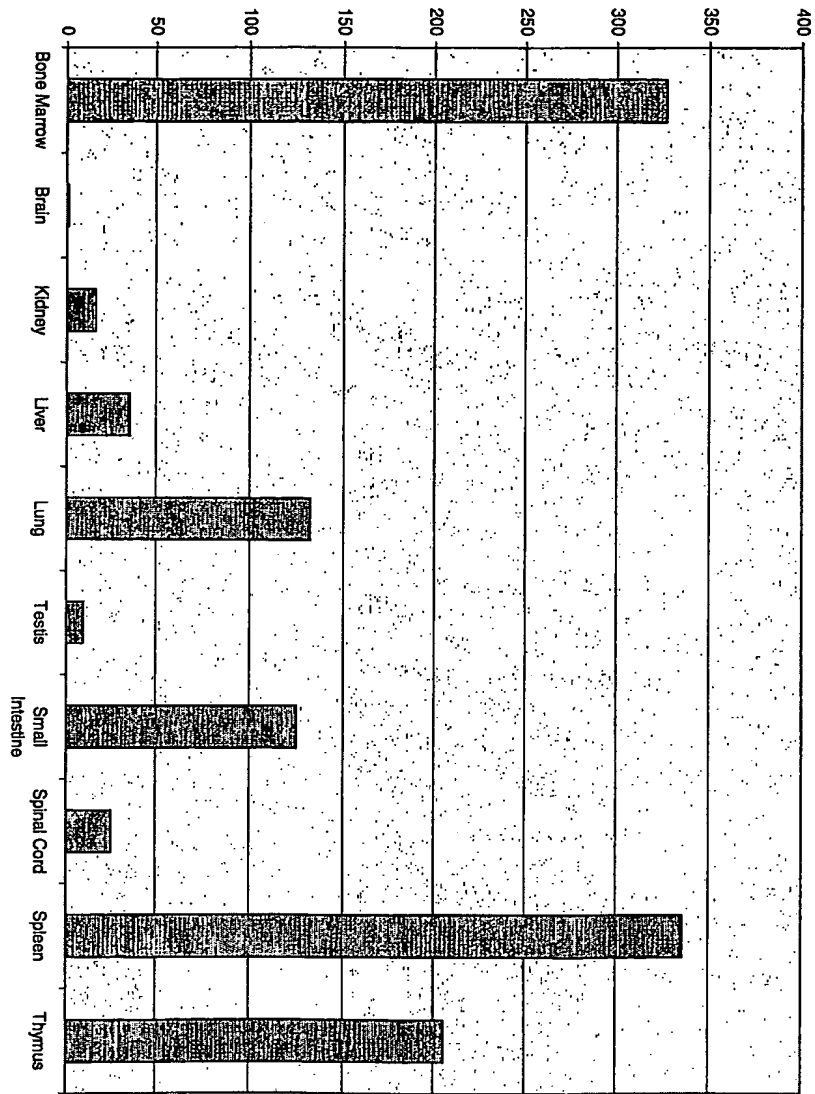


FIG. 5

CCACGCGTCCGGGCGCCAGGACCCTAGCGTGGCGCTCCAGCACCCCAGACCGTGGCGGGC
CCTCGCCTTAGGGAAGAGCAAGGGAAGAACTTTATTTGAACCGCGAACATTTTTTGGTCA
CTGAGATCGAGTCTCCAGTGCTTTGGCTTCCCGCCTCTTTATCTTGGGTTTGATCCCTG
AGCTGCTCTCCTTTCCCGAACCTCCCGGGGTGCAGCCTAGAGCCCTCCCGCGCGGCTGAC
TCCAGAGTAGAGGAAGGAGGCGGCTCCGGCTGGTCCCCGAAGCCCTCGCTGCCCCG
AGATGCGGATGGCCAGCAGTAGCGGGCGGTGGCCCCGCTCCCGGAGCGCACAGCA

ATGCAGGCGCTTAACATTACCCCGGAGCAGTTCTCTCGGCTGCTGCGGGACCACAACCTG
ACGCGGGAGCAGTTTCATCGCTCTGTACCGGCTGCGACCGCTCGTCTACACCCCAGAGCTG
CCGGGACGCGCAAGCTGGCCCTCGTGCTCACC GGCGTGCTCATCTTCGCCCTGGCGCTC
TTTGGCAATGCTCTGGTGTCTACGTGGTGACCCGAGCAAGGCCATGCGCACCGTCACC
AACATCTTTATCTGCTCCTTGGCGCTCAGTGACCTGCTCATCACCTTCTTCTGCATTCCC
GTCACCATGCTCCAGAACATTTCCGACAACCTGGCTGGGGGGTGCTTTTCATTTGCAAGATG
GTGCCATTTGTCCAGTCTACCGCTGTTGTGACAGAAATCCTCACTATGACCTGCATTGCT
GTGGAAGGACACAGGGACTTGTGCATCCTTTTAAATGAAGTGGAATACACCAACCGA
AGGGCTTTTACAAATGCTAGGTGTGGTCTGGCTGGTGCGAGTCATCGTAGGATCACCCATG
TGGCACGTGCAACAACCTTGAGATCAAATATGACTTCTTATATGAAAAGGAACACATCTGC
TGCTTAGAAGAGTGACAGCCCTGTGCACCAGAAGATCTACACCACCTTCATCCTTGTC
ATCCTCTTCTCCTGCTCTTATGGTGATGCTTATTCTGTACAGTAAAATTGGTTATGAA
CTTTGGATAAAGAAAAGAGTTGGGGATGGTTTCAGTGCTTCGAACTATTCATGGAAAAGAA
ATGTCCAAAATAGCCAGGAAGAAGAAACGAGCTGTCTATTATGATGGTGACAGTGGTGGCT
CTCTTTGCTGTGTGCTGGGCACCATTTCCATGTTGTCCATATGATGATTGAATACAGTAAT
TTTGAAAAGGAATATGATGATGTCACAATCAAGATGATTTTTTGCTATCGTGCAAAATATT
GGATTTTCCAACCTCCATCTGTAATCCCATTTGTCTATGCATTTATGAATGAAAACCTTCAAA
AAAAATGTTTTGTCTGCAGTTTGTATTGTCATAGTAAATAAAACCTTCTCTCCAGCACAA
AGGCATGGAAATTCAGGAATTACAATGATGCGGAAGAAAGCAAAGTTTTCCCTCAGAGAG
AATCCAGTGGAGGAACCAAAGGAGAAGCATTTCAGTGATGGCAACATTGAAGTCAAATTG
TGTGAACAGACAGAGGAGAAGAAAAAGCTCAAACGACATCTTGCTCTCTTTAGGTCTGAA
CTGGCTGAGAATTCTCCTTTAGACAGTGGGCATTAA

TTATAACAATATCTTCATAATTAATGCCCTTCAGATTGTAACCCAAAGAGAAAAATTATTT
TGAGCAAAGGTCAAATACTCTTTTATTCTTAAGATGATGACAAGAAGAAAACAAATCAT
GTTTCCATTAAAAAATGACACGAGGCTAGTCCAAGTGCAGTGATGTTTACAACCAATTGA
TCACAATCATTTAACAGATTTCTGTGTTTCTCTCATTTCCCACTGCTTCACTTGACTAGC
CTTAAAAAAGCAACATGGAAGGCCAGGCACGGTGGCTCATGCCTGTAATCCAGCACTTT
GGGAGGCCTAGACGGGCGGATCACGAGGTGAGGATCAAAACCATCCTGGCTAACACGG
TGAAACCCCATCTCTGCTAAAAATACAAAAATTAGCCGGGCGTGTTGGCGGGCACCTGTA
GTCCAGCTACTTTGGGAGCCTCAGGCGGGAGAATGGTGTGAACCCGGGAGGCGGAGCTTG
CAGTGATCCGAGATCGTGCCACTGCACTCCAGCCTGGGCGAAAGAGCGAGACTCCCCGTC
TCAAAAAAATTTTTTTGAAAAATTCGTAAACCATACTTTTAAGATTATTTCAAGTGATT
TTTAAAAATCTTGTACAGAAATCAGGGTCTTTAGCTAGCAGTTTTTCTCCACGCAGTCA
CTGTAATGTGACTATGTATTGCTAGATTGAATAAGAAAAATAAATATCTTCTTCCCTT
GAAAAAAGAAAAAAGAAAAAAGGCGGCGCTCTAGAGGATCCCTC
GAGGGGCCCAAGCT

FIG. 6

MQALNITPEQFSRLLRDHNLTREQFIALYRLRPLVYTPELPGRAKLALVLTGVLIFALAL
FGNALVFYVVTRSKAMRTVTNIFICSLALSDLLITFFCIPVTMLQNISDNWLGGAFICKM
VPFVQSTAVVTEILTMTCIAVERHQGLVHPFKMKWQYTNRRRAFTMLGVVWLVAIVIGSPM
WHVQOLEIKYDFLYEKEHICCLEEWTSPVHQKIYTTFILVILFLLPLMVMLILYSKIGYE
LWIKKRVGDGSVLRTIHGKEMSKIARKKKRAVIMMVTVVALFAVCWAPFHVVHMMIEYSN
FEKEYDDVTIKMIFAIVQIIIGFSNSICNPVYA FMNENFKKNVLSAVCYCIVNKTFSPAQ
RHGNSGITMMRKKAKFSLREN PVEETKGEAFSDGNIEVKLCEQTEKKKLKRHLALFRSE
LAENSPLDSGH

FIG. 7

1 MQALNITPEQ FSRLLRDHNL TREQFIALYR LRPLVYTPEL PGRAKLALVL
51 TGVLFIFALAL FGNALVFYVV TRSKAMRTVT NIFICSLALS DLLITFFCIP
101 VTMLQNISDN WLGGAFICKM VPFVQSTAVV TEILTMTCIA VERHQGLVHP
151 FKMKWQYTNR RAFTMLGVVW LVAVIVGSPM WHVQQLEIKY DFLYEKEHIC
201 CLEEWTSPPVH QKIYTTFILV ILFLLPLMVM LILYSKIGYE LWIKKRVGDG
251 SVLRTIHGKE MSKIARKKKR AVIMMVTVVA LFAVCWAPFH VVHMMIEYSN
301 FEKEYDDVTI KMIFAIVQII GFSNSICNPI VYAFMNENFK KNVLSAVCYC
351 IVNKTFSAPQ RHGNSGITMM RKKAKFSLRE NPVEETKGEA FSDGNIEVKL
401 CEQTEEEKKL KRHLALFRSE LAENSPLDSG H

Transmembrane Prediction for the Novel Human GPCR, HGPRBM2

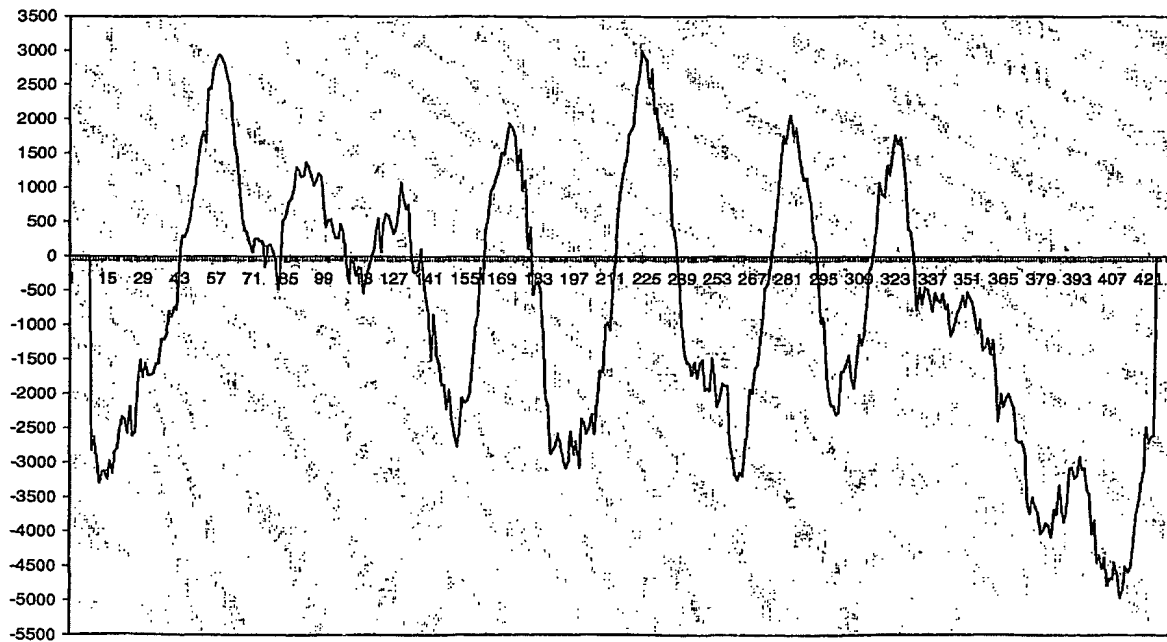


FIG. 8

```

OX2R_HUMAN  ~~~~~
OX2R_RAT    ~~~~~
NY4R_MOUSE  ~~~~~
NY4R_RAT    ~~~~~
NY6R_RABIT  ~~~~~
Q9WVD0      ~~~~~
O57463      ~~~~~
NY2R_HUMAN  ~~~~~
Q9Y5X5      MNSFFGTPAASWCLLESDVSSAPDKEAGRERRALSVQQRGGPAWSGSLEWSRQSAGDRRR
HGPRBMY2    ~~~~~
GALR_MOUSE  ~~~~~

OX2R_HUMAN  ~~~~~MSGTKLEDSPPCRNWSSASENET
OX2R_RAT    ~~~~~MSSTKLEDSLPRRWSSASENET
NY4R_MOUSE  ~~~~~MNTSHFLAPFPFG
NY4R_RAT    ~~~~~MNTSHLMASLSPA
NY6R_RABIT  ~~~~~MEVSLNDP
Q9WVD0      ~~~~~MNSTSFQLEN
O57463      ~~~~~
NY2R_HUMAN  ~~~~~MGPIGAEADENQTV EEMKVEQYGPQTTPRGEL
Q9Y5X5      LGLSRQTAKSSWSRSDRTCCCRRAWWILVPAADRARRERFIMNEKWDTNSSSENWHP IWN
HGPRBMY2    ~~~~~MQALNITPEQFSRLLR
GALR_MOUSE  ~~~~~MELA

OX2R_HUMAN  QEPFLNPIDYDDEELRLWLREYLHKEYEWWLIAGYIIFVVALIGNV.LVCVAW..K
OX2R_RAT    QEPFLNPIDYDDEELRLWLREYLHKEYEWWLIAGYIIFVVALIGNV.LVCVAW..K
NY4R_MOUSE  SLQKNGINPLDSPX.NL.SDGCQDSAELLAFTITTYSHETILGLGNLCIT.FVTTROK
NY4R_RAT    FLQKNGINPLDSL.NL.SDGCQDSADLLAFITTYSVETVLGLGNLCIT.FVTTROK
NY6R_RABIT  ASNKTSAKSNSSAF.YYES..CQSESLALLLTAYTVVLMGTCGNLSLITITFKOR
Q9WVD0      HSVHYNLSEKPSPE.AEENDDCHLELAVIFTALAYGAMITLGVSGNLALILIII.KOK
O57463      ~~~~MERGHLNNSW.LLEDPTCPASLSSTFTLVAYSTMLAVGLVNTCLVVVL.TROK
NY2R_HUMAN  VPD.PEPELIDSTKL.I.....EVQVLLAVCSHILLGVGN.SLVITHVILKFK
Q9Y5X5      VND.TKHLYSDINI.TYVNYLHQE.QVAAFTHSYFTIFFLCMMGN.TVVCFTVMRNK
HGPRBMY2    DHNLTREQFIALYRL.RPLVYTPELEGRAKLAELVTGVLEFALALGN.ALVFYVTRSR
GALR_MOUSE  MVNLSSENGSDPEPP.APESRPLFGIGVENFILTAVFGLIFAMGVGN.SLVITILARSK

OX2R_HUMAN  NHHMRTVTNYFIVNLSLADVLVITICLPATLVVDITETWFFGQSLCKVIEYLOTVSVS
OX2R_RAT    NHHMRTVTNYFIVNLSLADVLVITICLPATLVVDITETWFFGQSLCKVIEYLOTVSVS
NY4R_MOUSE  ..EKSNTNLLIANLAFSDFLMCLICQPLTVTYTIMDYWIFGEVLCKMITFIQCMSVVS
NY4R_RAT    ..EKSNTNLLIANLAFSDFLMCLICQPLTVTYTIMDYWIFGEVLCKMITFIQCMSVVS
NY6R_RABIT  ..EAQNTNLLIANLSLSDILVCMCIPFTADYTLMDRWIFGNTMCKLTSYVQSVSISVS
Q9WVD0      ..EMRNVTNLLIVNLSFSDLLVALMCLPFTFYVTLMDHWIFGEIMCKLNEFVCCVSIVS
O57463      ..EMRNVTNLLIVNLSFSDILVCLVCLPVTLTYTMDRWILGEALCKVTPFVQCMSVIVS
NY2R_HUMAN  ..SMRTVTNFFIANLAVADLLVNTICLPFTLTITLMGEWKMGVPLCHELVEYAOGLAVQVS
Q9Y5X5      ..HMHTVTNFFIANLAFSDLLVGTFCMPITLEDNIIAGWPFQNTMCKISGLVQGSVAAS
HGPRBMY2    ..AMRTVTNFFICSLAFSDLLITPFCIPVETMLQNSDNMLGGAFICKMVEFFVQSTAVTE
GALR_MOUSE  PGKPRSTTNLLFIVNLSLADLAYILFCIPFQATVYALPTWVLGAFICKFHHFFTVSMIVS

```

FIG. 9

OX2R_HUMAN VLTLSCTALDRWYAICHPLM..FNSTAKRARNSTVITIVIVSCITMIPQATVMECSTVFPFG
 OX2R_RAT VLTLSCTALDRWYAICHPLM..FNSTAKRARNSTVITIVIVSCITMIPQATVMERSSSLPG
 NY4R_MOUSE ILSLVLVALERHQLIINP..TGWKPSIFOAYLCIVVIWIFSCFLSLPFLANSIENDLFHY
 NY4R_RAT ILSLVLVALERHQLIINP..TGWKPSISOAYLCIVVIWIFSCFLSLPFLANSIENDLFHY
 NY6R_RABIT ILSLVLIATERYQLIVNP..RGWKPSASHAYWSTMLIWLFLSLISTPLLSYHITDEPER
 Q9WVD0 ILSLVLIATERYHQLIINP..RCWRPNNRHAYICIAVIWVLAVASSLPFMBIYQVLTDEPQ
 O57463 ILSMVLIALERHQLIHP..TGWKPVVRHSYLAVAVIWLACFLSLPFLSFNITNSPEH
 NY2R_HUMAN TITLTIVIALDRHRCIVYHLESK..ISKRISTLIGLAWGISALLASPLATF....REYS
 Q9Y5X5 VFTLVATAVDREFQCVVYPPFKPK..LTIKTAFTVITMIWVLATITMSPSAVMLHVQEEKY
 HGPRBMY2 ILTMTCTIAVERHQLVHPFKMKQOYENRRATMIGVVLVAVIVGSP...MWHVQOLE..
 GALR_MOUSE IFTLAANSVDRYVAIVHSRRSSSLRVSRNALGCVGFIWALSAMASPVAYHQRI....FH

OX2R_HUMAN LANKTTLE.....TVCDERWGGG...IYPKMYHICFFLVITVMAPICLMVLALQIHRKL
 OX2R_RAT LANKTTLE.....TVCDERWGGG...IYPKMYHICFFLVITVMAPICLMVLALQIHRKL
 NY4R_MOUSE NHSKVVEFLEDKVV..CFVSWSSDHHRLIYTTFL...LLFOYCIPLAFILVCYIRIYQRL
 NY4R_RAT NHSKVVEFLEDKVV..CFVSWSSDHHRLIYTTFL...LLFOYCIPLAFILVCYIRIYQRL
 NY6R_RABIT NLSLPTDLYSHHVV..CVEHWPSKTNQLIYSTSL...LMLQYFVPLGFMFICVYLKIVICL
 Q9WVD0 NYTL..DAFKDKLV..CFDQPSDSHRLSYTTLL...LVLOVFGPLCFIFTCYFKIVIRL
 O57463 NLSLPFNPFSDFET..CIEQWPSEGNNLYTTTL...LLCOYCIPLALILVCYIRIFLRL
 NY2R_HUMAN LHEIIPDE...IVACTEKWPGEE..KSIYGTVYSLSSLLILVPLGLISFSYTRISKL
 Q9Y5X5 RRLNLSQNKTSPIVWOREDWENQEMKIIYTTVEFAN...IYLAPLSLIVIMYGRIGISL
 HGPRBMY2 .IKYDFLEKEHEI..CCLEENISPVHQKIYTTFL...LVILPLPLMLVMLILYSKIGYEL
 GALR_MOUSE R.....DSNQTFCEWQWENKLHKAY....VVCTTFMGYLLPLLLICFCYAKVLNHL

OX2R_HUMAN WCQIPGTSSVQORKWKLPQVSPQPRGPGQPTKSRMSAVAAEIKQIRARRKTARMLMVVL
 OX2R_RAT WCQIPGTSSVQORKWKQPQVSPQPRGSGQQSKARISAVAAEIKQIRARRKTARMLMVVL
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 NY6R_RABIT HERN.....SKIDRR.....RENE.SRLT.....E.NKRINTMLISTIV
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 O57463 SERK.....DAVERA.....RGGK.OKKA.....KGSKRYNAMLASIV
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 HGPRBMY2 WIKRVGDGSLRTI.....HGKEMSKTA.....RKKKRAVIMVTVV
 GALR_MOUSE HKRLK.....NMSKKSEAS.....K...KKTAQTVLVVV

OX2R_HUMAN LVFAICVLEPISLNLKRVFGMFAHTEDRETVEYAWFTFS..EWLVYANSAANPIIYNFLSG
 OX2R_RAT LVFAICVLEPISLNLKRVFGMFTHTEDRETVEYAWFTFS..EWLVYANSAANPIIYNFLSG
 NY4R_MOUSE TAFAYLWLPPLHVFNTLEDWYQ...EATPACHGNLIFLM..CELTAMASTCVNPFYIGFLNI
 NY4R_RAT TAFAYLWLPPLHVFNTLEDWYQ...EATPACHGNLIFLM..CELTAMASTCVNPFYIGFLNI
 NY6R_RABIT VTFAACWLPLNTFNVFDFWYH...EVLMSCHDLVFAI..CELVAMVSTCINPLFYGFLNR
 Q9WVD0 MAFAVCWLPPLNTFNTVFDWNH...QITATCNHNLLELT..CELTAMISTCVNPIFYGFLNK
 O57463 AAFALCWLPPLNVFNTTFDWNH...EATPVCQHDATFSA..CELTAMASTCVNPFYIGFLNN
 NY2R_HUMAN VVFAVSWLPPLHAFQAVD..IDSQVLDLKEYKL..IFTV..FELTAMCSTFANPLLYGWMNS
 Q9Y5X5 ELFILSWLPPLWTLMMLSDYADLSPNELQIINI..YIYPPF..AHWLAFQNSSVNPIIYGFENE
 HGPRBMY2 ALFAVCWAFPHVHMIEYSNFE..KEYDDVTIKMIFAN..VQITGFSNSICNPITYAFMNE
 GALR_MOUSE VVFGISWLBHHVYHL...WAEFG..AFPLTPASFFFRITAFCLAYSNSSVNPIIYAFISE

FIG. 9 Continued

```

OX2R_HUMAN KRRDEFKAAFSCCCLGVHHRQE..DRLTRGRTSTESRKSLTTQISNFDNISKLSEQVVL
OX2R_RAT   KRRDEFKAAFS.CCLGVHRRQG..DRLARGRTSTESRKSLTTQISNFDNVSKLSEHVALT
NY4R_MOUSE NEKKDIKALVLTCHC.RSPQGES.EHHPLSTVHTDLSKGSMMRGSKSNFI~~~~~
NY4R_RAT   NEKKDIKALVLTCHC.RPPQGEF.EPLPLSTVHTDLSKGSMMRGSKSNVM~~~~~
NY6R_RABIT NFQKDLVVLIIHCLC.FALR.ERYEMHAISTLHTDESKGST...RVAHIPAGI~~~~~
Q9WVD0     NFQKDLVVLIIHCLC.D.FRSRDDYETIAMSHHTLVSKTST...KQAS.PLAFKKISCVE
O57463     NFQKDLVVLIIHCLC.WG.PAESYESFPLSTVSTGITRGSILSNGSASTYQPHKKNSLEQ
NY2R_HUMAN NYRKAFLSAFR...C..EORLDAI..HSEVSYA..FKAKKNLEVRKNSGPNDSETEATNV~
Q9Y5X5     NFRRGFQEAFLQLC..QKRAKPM..E...AYE.LKAKSHMLI..NTS.NQLVQESTFQN
HGPRBMY2   NEKKNYLSAVCYQIV..NKTFSIPAQRHGNSGLTMMRKRAKPSLREN..PVEETKGEAFSD
GALR_MOUSE NFRKAYKQVEKCHVQDESPRSETKENKSRMDTPPSTNCTHY~~~~~

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OX2R_HUMAN SISTLPAANGAGPLQNW~~~~~
OX2R_RAT   SISTLPAANGAGPLQNWYLQQGVPSLLSTWLEV~~~~
NY4R_MOUSE ~~~~~
NY4R_RAT   ~~~~~
NY6R_RABIT ~~~~~
Q9WVD0     NEKI~~~~~
O57463     KESI~~~~~
NY2R_HUMAN ~~~~~
Q9Y5X5     PHGETLLYRKSAEKPQQELVMEELK.ETTNSEI~~~~
HGPRBMY2   GNIEVKLCSEQTEKKKLRHLALFRSELAENSPLDSCG
GALR_MOUSE ~~~~~

```

FIG. 9 Continued

FIG. 10

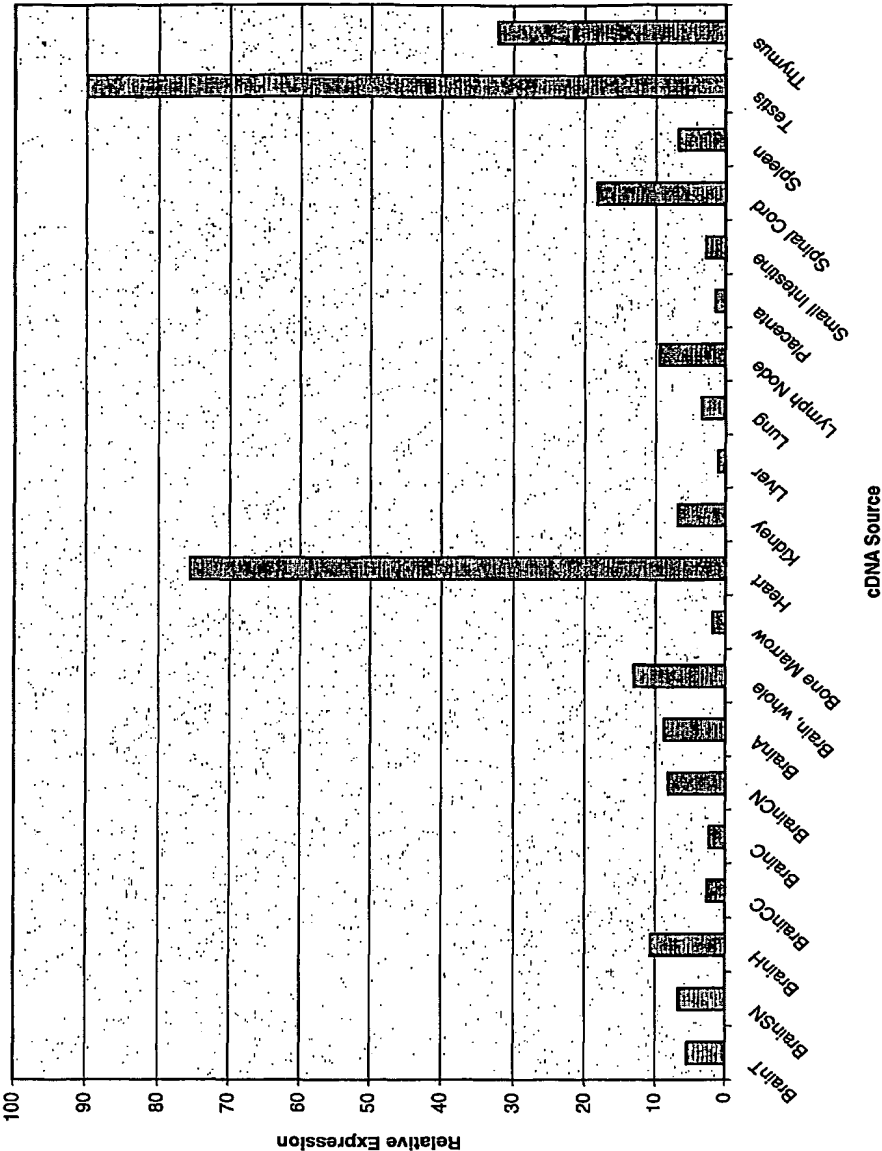


Figure 11

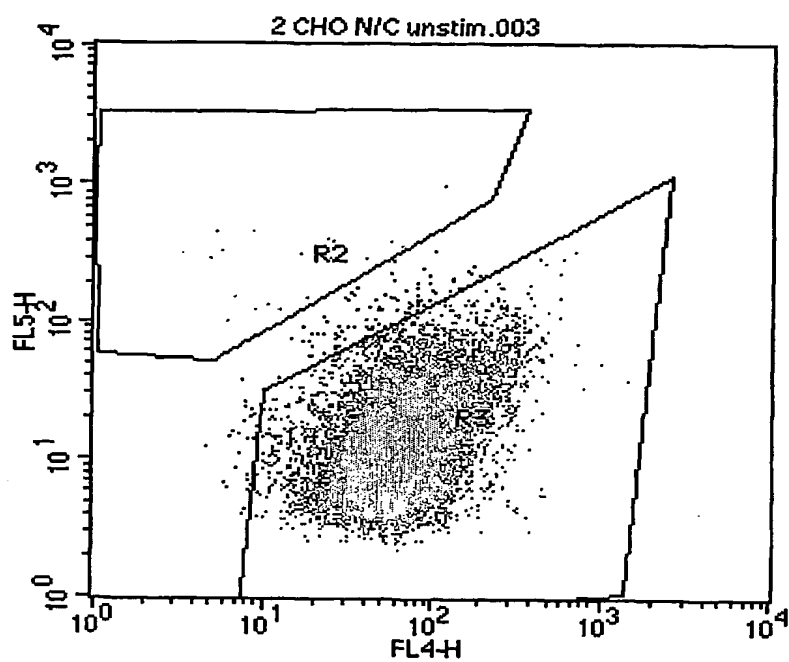


Figure 12

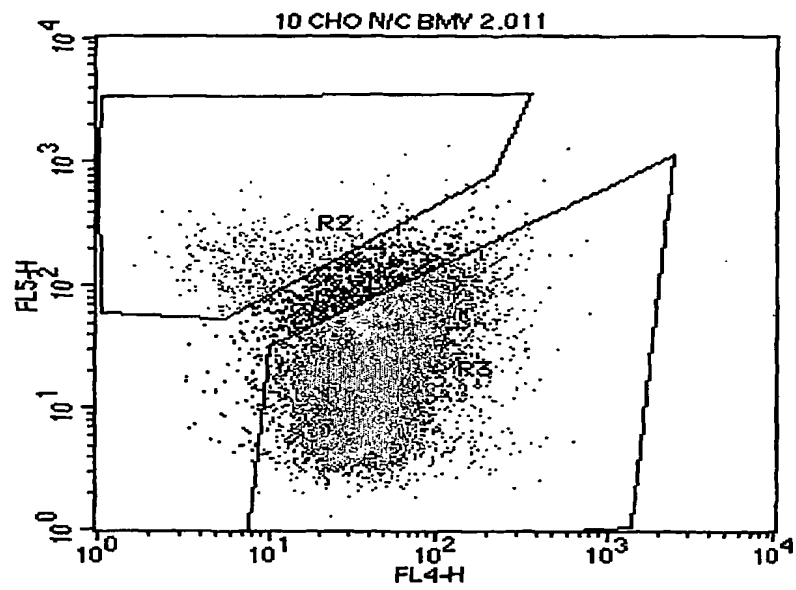


Figure 13

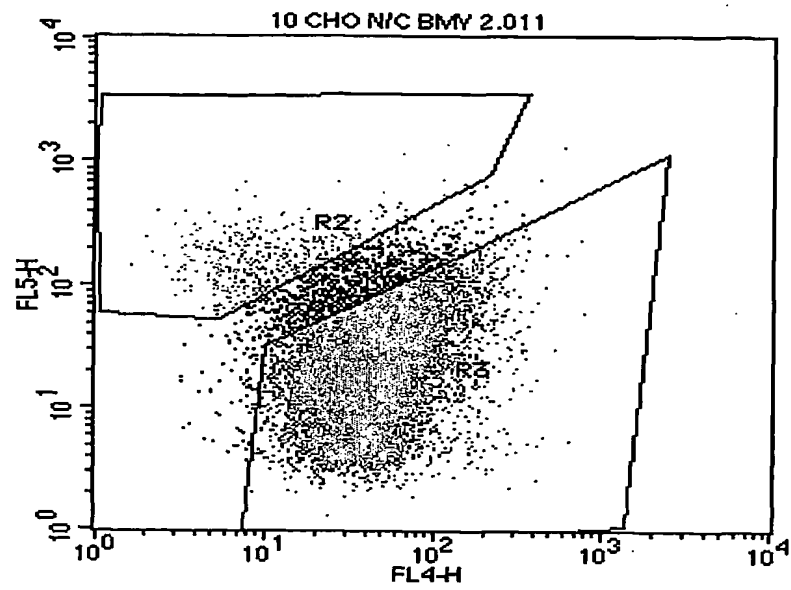


Figure 14

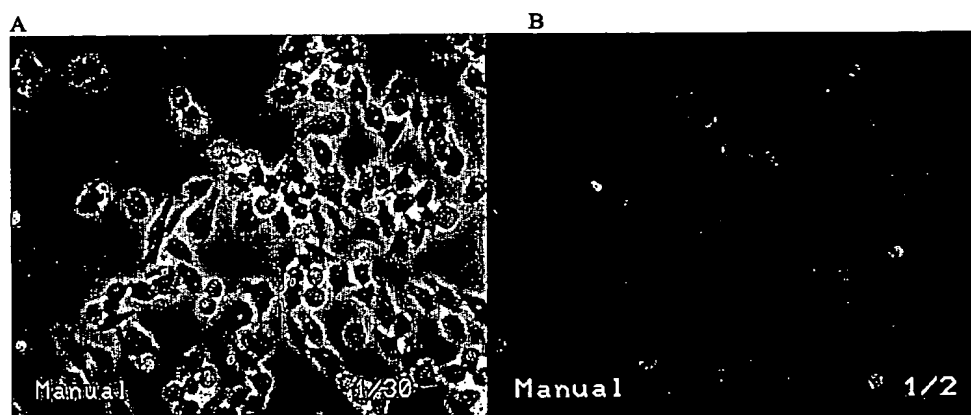
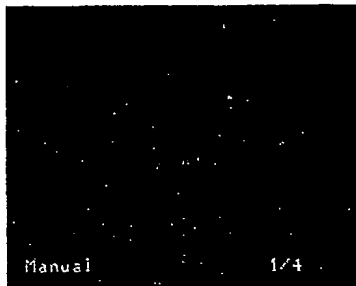


Figure 15

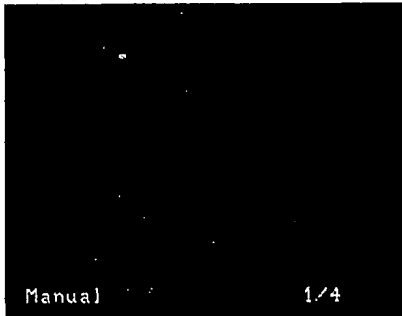
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c. Cho-NFAT CRE HGPRBMY2 Intermediate



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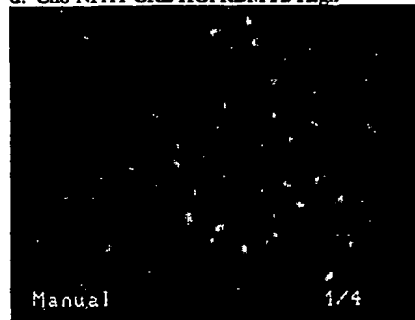
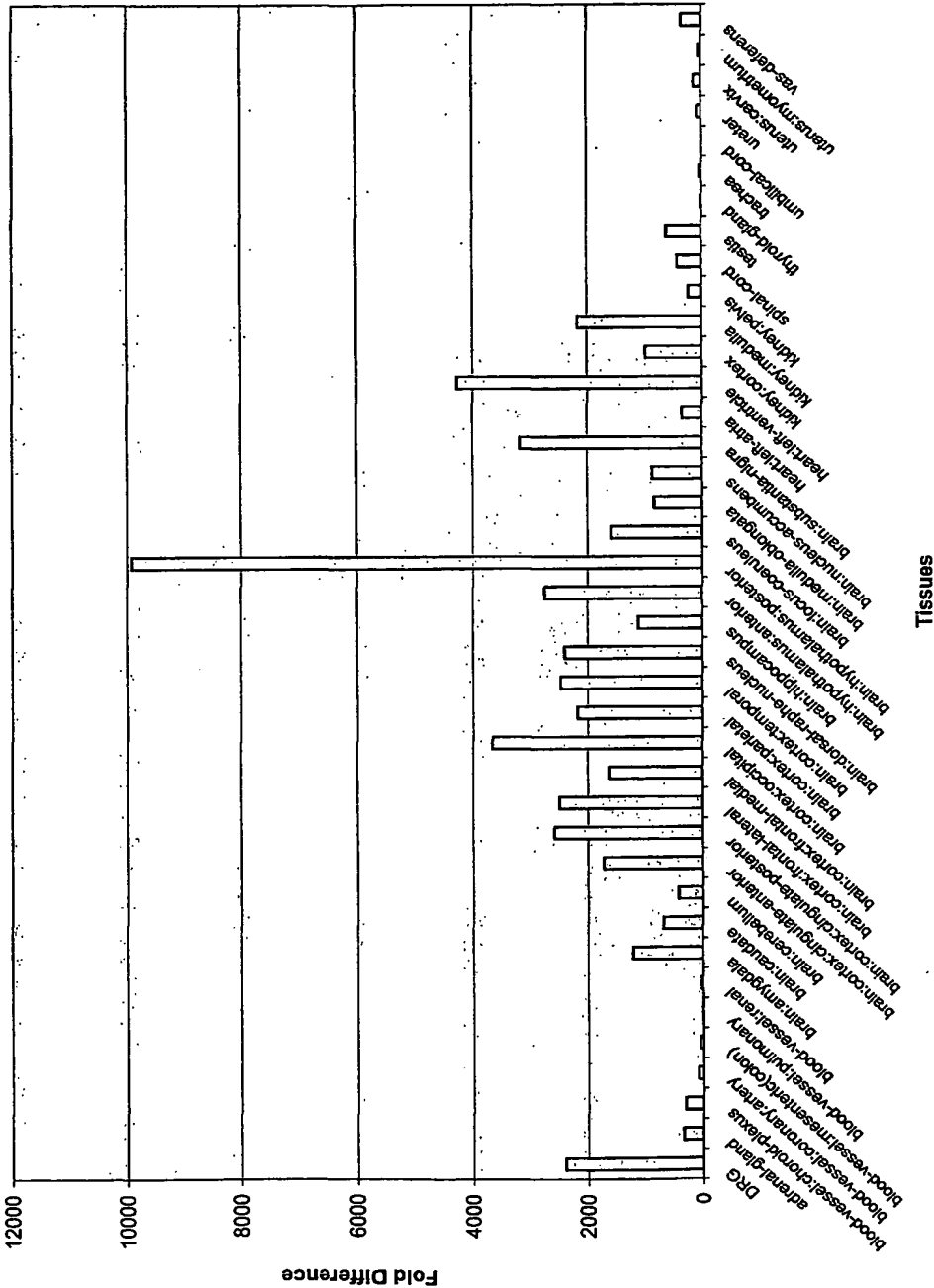


Figure 16



SEQUENCE LISTING

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<130> D0132 PCT

<150> US 60/270,793

<151> 2001-02-23

<150> US 60/270,792

<151> 2001-02-23

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<151> 2001-06-06

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 35 40 45
 Cys Arg Arg Met Gly Pro Arg Ser Pro Ser Val Ile Phe Met Ile Asn
 50 55 60
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 Tyr Tyr His Cys Asn Arg His His Trp Val Phe Gly Val Leu Leu Cys
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Thr Met Thr Cys Ile Ser Val Glu Arg Phe Leu Gly Val Leu Tyr Pro
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 Met Gln Ala Leu Asn Ile Thr Pro Glu Gln Phe Ser Arg Leu Leu Arg
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Pro	Leu	Val	Tyr	Thr	Pro	Glu	Leu	Pro	Gly	Arg	Ala	Lys	Leu	Ala	Leu		
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gtg	ctc	acc	ggc	gtg	ctc	atc	ttc	gcc	ctg	gcg	ctc	ttt	ggc	aat	gct	550	
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ctg	gtg	ttc	tac	gtg	gtg	acc	cgc	agc	aag	gcc	atg	cgc	acc	gtc	acc	598	
Leu	Val	Phe	Tyr	Val	Val	Thr	Arg	Ser	Lys	Ala	Met	Arg	Thr	Val	Thr		
65					70					75					80		
aac	atc	ttt	atc	tgc	tcc	ttg	gcg	ctc	agt	gac	ctg	ctc	atc	acc	ttc	646	
Asn	Ile	Phe	Ile	Cys	Ser	Leu	Ala	Leu	Ser	Asp	Leu	Leu	Ile	Thr	Phe		
				85					90					95			
ttc	tgc	att	ccc	gtc	acc	atg	ctc	cag	aac	att	tcc	gac	aac	tgg	ctg	694	
Phe	Cys	Ile	Pro	Val	Thr	Met	Leu	Gln	Asn	Ile	Ser	Asp	Asn	Trp	Leu		
			100					105					110				
ggg	ggt	gct	ttc	att	tgc	aag	atg	gtg	cca	ttt	gtc	cag	tct	acc	gct	742	
Gly	Gly	Ala	Phe	Ile	Cys	Lys	Met	Val	Pro	Phe	Val	Gln	Ser	Thr	Ala		
		115					120					125					
gtt	gtg	aca	gaa	atc	ctc	act	atg	acc	tgc	att	gct	gtg	gaa	agg	cac	790	
Val	Val	Thr	Glu	Ile	Leu	Thr	Met	Thr	Cys	Ile	Ala	Val	Glu	Arg	His		
		130				135					140						
cag	gga	ctt	gtg	cat	cct	ttt	aaa	atg	aag	tgg	caa	tac	acc	aac	cga	838	
Gln	Gly	Leu	Val	His	Pro	Phe	Lys	Met	Lys	Trp	Gln	Tyr	Thr	Asn	Arg		
145					150					155					160		
agg	gct	ttc	aca	atg	cta	ggt	gtg	gtc	tgg	ctg	gtg	gca	gtc	atc	gta	886	
Arg	Ala	Phe	Thr	Met	Leu	Gly	Val	Val	Trp	Leu	Val	Ala	Val	Ile	Val		
				165					170					175			
gga	tca	ccc	atg	tgg	cac	gtg	caa	caa	ctt	gag	atc	aaa	tat	gac	ttc	934	
Gly	Ser	Pro	Met	Trp	His	Val	Gln	Gln	Leu	Glu	Ile	Lys	Tyr	Asp	Phe		
			180					185					190				
cta	tat	gaa	aag	gaa	cac	atc	tgc	tgc	tta	gaa	gag	tgg	acc	agc	cct	982	
Leu	Tyr	Glu	Lys	Glu	His	Ile	Cys	Cys	Leu	Glu	Glu	Trp	Thr	Ser	Pro		
		195					200					205					
gtg	cac	cag	aag	atc	tac	acc	acc	ttc	atc	ctt	gtc	atc	ctc	ttc	ctc	1030	
Val	His	Gln	Lys	Ile	Tyr	Thr	Thr	Phe	Ile	Leu	Val	Ile	Leu	Phe	Leu		
		210					215					220					
ctg	cct	ctt	atg	gtg	atg	ctt	att	ctg	tac	agt	aaa	att	ggt	tat	gaa	1078	
Leu	Pro	Leu	Met	Val	Met	Leu	Ile	Leu	Tyr	Ser	Lys	Ile	Gly	Tyr	Glu		
225					230					235					240		
ctt	tgg	ata	aag	aaa	aga	gtt	ggg	gat	ggt	tca	gtg	ctt	cga	act	att	1126	
Leu	Trp	Ile	Lys	Lys	Arg	Val	Gly	Asp	Gly	Ser	Val	Leu	Arg	Thr	Ile		

245										250					255					
cat	gga	aaa	gaa	atg	tcc	aaa	ata	gcc	agg	aag	aag	aaa	cga	gct	gtc	1174				
His	Gly	Lys	Glu	Met	Ser	Lys	Ile	Ala	Arg	Lys	Lys	Lys	Arg	Ala	Val					
			260					265					270							
att	atg	atg	gtg	aca	gtg	gtg	gct	ctc	ttt	gct	gtg	tgc	tgg	gca	cca	1222				
Ile	Met	Met	Val	Thr	Val	Val	Ala	Leu	Phe	Ala	Val	Cys	Trp	Ala	Pro					
			275				280					285								
ttc	cat	gtt	gtc	cat	atg	atg	att	gaa	tac	agt	aat	ttt	gaa	aag	gaa	1270				
Phe	His	Val	Val	His	Met	Ile	Ile	Glu	Tyr	Ser	Asn	Phe	Glu	Lys	Glu					
			290			295					300									
tat	gat	gat	gtc	aca	atc	aag	atg	att	ttt	gct	atc	gtg	caa	att	att	1318				
Tyr	Asp	Asp	Val	Thr	Ile	Lys	Met	Ile	Phe	Ala	Ile	Val	Gln	Ile	Ile					
			305			310				315					320					
gga	ttt	tcc	aac	tcc	atc	tgt	aat	ccc	att	gtc	tat	gca	ttt	atg	aat	1366				
Gly	Phe	Ser	Asn	Ser	Ile	Cys	Asn	Pro	Ile	Val	Tyr	Ala	Phe	Met	Asn					
				325				330						335						
gaa	aac	ttc	aaa	aaa	aat	gtt	ttg	tct	gca	gtt	tgt	tat	tgc	ata	gta	1414				
Glu	Asn	Phe	Lys	Lys	Asn	Val	Leu	Ser	Ala	Val	Cys	Tyr	Cys	Ile	Val					
			340					345					350							
aat	aaa	acc	ttc	tct	cca	gca	caa	agg	cat	gga	aat	tca	gga	att	aca	1462				
Asn	Lys	Thr	Phe	Ser	Pro	Ala	Gln	Arg	His	Gly	Asn	Ser	Gly	Ile	Thr					
			355				360					365								
atg	atg	cgg	aag	aaa	gca	aag	ttt	tcc	ctc	aga	gag	aat	cca	gtg	gag	1510				
Met	Met	Arg	Lys	Lys	Ala	Lys	Phe	Ser	Leu	Arg	Glu	Asn	Pro	Val	Glu					
			370				375					380								
gaa	acc	aaa	gga	gaa	gca	ttc	agt	gat	ggc	aac	att	gaa	gtc	aaa	ttg	1558				
Glu	Thr	Lys	Gly	Glu	Ala	Phe	Ser	Asp	Gly	Asn	Ile	Glu	Val	Lys	Leu					
			385			390				395					400					
tgt	gaa	cag	aca	gag	gag	aag	aaa	aag	ctc	aaa	cga	cat	ctt	gct	ctc	1606				
Cys	Glu	Gln	Thr	Glu	Glu	Lys	Lys	Lys	Leu	Lys	Arg	His	Leu	Ala	Leu					
			405						410					415						
ttt	agg	tct	gaa	ctg	gct	gag	aat	tct	cct	tta	gac	agt	ggg	cat		1651				
Phe	Arg	Ser	Glu	Leu	Ala	Glu	Asn	Ser	Pro	Leu	Asp	Ser	Gly	His						
			420					425					430							
taattataac	aatatcttca	taattaatgc	ccttcagatt	gtaacccaaa	gagaaaatta											1711				
ttttgagcaa	aggtcaaata	ctctttttat	tcttaagatg	atgacaagaa	gaaaacaaat											1771				
catgtttcca	ttaaaaaatg	acacgaggct	agtccaagtg	cagtgatggt	tacaaccaat											1831				
tgatcacaat	catttaacag	attttctgtgt	tccttctcat	tcccactgct	tcacttgact											1891				
agccttaaaa	aagcaacatg	gaaggccagg	cacggtggct	catgcctgta	atcccagcac											1951				
tttgggaggg	ctagacgggc	ggatcacgag	gtcaggagat	caaaaccatc	ctggctaaca											2011				

cgggtgaaacc ccactctctgc taaaaataca aaaattagcc gggcgtggtg gcgggcacct 2071
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 gtctcaaaaa aaattttttt gaaaaattcg taaaccatac ttttaagatt atttcagtgg 2251
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 tcactgtaat gtgactatgt attgctagat tgaataagaa aataaaataa tatcttcttc 2371
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 ctcgaggggc ccaagct 2448

<210> 17
 <211> 431
 <212> PRT
 <213> Homo sapiens

<400> 17

Met Gln Ala Leu Asn Ile Thr Pro Glu Gln Phe Ser Arg Leu Leu Arg
 1 5 10 15

Asp His Asn Leu Thr Arg Glu Gln Phe Ile Ala Leu Tyr Arg Leu Arg
 20 25 30

Pro Leu Val Tyr Thr Pro Glu Leu Pro Gly Arg Ala Lys Leu Ala Leu
 35 40 45

Val Leu Thr Gly Val Leu Ile Phe Ala Leu Ala Leu Phe Gly Asn Ala
 50 55 60

Leu Val Phe Tyr Val Val Thr Arg Ser Lys Ala Met Arg Thr Val Thr
 65 70 75 80

Asn Ile Phe Ile Cys Ser Leu Ala Leu Ser Asp Leu Leu Ile Thr Phe
 85 90 95

Phe Cys Ile Pro Val Thr Met Leu Gln Asn Ile Ser Asp Asn Trp Leu
 100 105 110

Gly Gly Ala Phe Ile Cys Lys Met Val Pro Phe Val Gln Ser Thr Ala
 115 120 125

Val Val Thr Glu Ile Leu Thr Met Thr Cys Ile Ala Val Glu Arg His
 130 135 140
 Gln Gly Leu Val His Pro Phe Lys Met Lys Trp Gln Tyr Thr Asn Arg
 145 150 155 160
 Arg Ala Phe Thr Met Leu Gly Val Val Trp Leu Val Ala Val Ile Val
 165 170 175
 Gly Ser Pro Met Trp His Val Gln Gln Leu Glu Ile Lys Tyr Asp Phe
 180 185 190
 Leu Tyr Glu Lys Glu His Ile Cys Cys Leu Glu Glu Trp Thr Ser Pro
 195 200 205
 Val His Gln Lys Ile Tyr Thr Thr Phe Ile Leu Val Ile Leu Phe Leu
 210 215 220
 Leu Pro Leu Met Val Met Leu Ile Leu Tyr Ser Lys Ile Gly Tyr Glu
 225 230 235 240
 Leu Trp Ile Lys Lys Arg Val Gly Asp Gly Ser Val Leu Arg Thr Ile
 245 250 255
 His Gly Lys Glu Met Ser Lys Ile Ala Arg Lys Lys Lys Arg Ala Val
 260 265 270
 Ile Met Met Val Thr Val Val Ala Leu Phe Ala Val Cys Trp Ala Pro
 275 280 285
 Phe His Val Val His Met Met Ile Glu Tyr Ser Asn Phe Glu Lys Glu
 290 295 300
 Tyr Asp Asp Val Thr Ile Lys Met Ile Phe Ala Ile Val Gln Ile Ile
 305 310 315 320
 Gly Phe Ser Asn Ser Ile Cys Asn Pro Ile Val Tyr Ala Phe Met Asn
 325 330 335
 Glu Asn Phe Lys Lys Asn Val Leu Ser Ala Val Cys Tyr Cys Ile Val
 340 345 350
 Asn Lys Thr Phe Ser Pro Ala Gln Arg His Gly Asn Ser Gly Ile Thr

355 360 365
 Met Met Arg Lys Lys Ala Lys Phe Ser Leu Arg Glu Asn Pro Val Glu
 370 375 380
 Glu Thr Lys Gly Glu Ala Phe Ser Asp Gly Asn Ile Glu Val Lys Leu
 385 390 395 400
 Cys Glu Gln Thr Glu Glu Lys Lys Lys Leu Lys Arg His Leu Ala Leu
 405 410 415
 Phe Arg Ser Glu Leu Ala Glu Asn Ser Pro Leu Asp Ser Gly His
 420 425 430

<210> 18
 <211> 21
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<210> 19
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 <212> DNA
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<400> 19
 acagggtgg tccactcttc t 21

<210> 20
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 cccaagctta tgcaggcgct taacattacc ccg 33

<210> 21
 <211> 32
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<400> 21
 cgggatcctt aatgccactg tctaaaggaa ga 32

<210> 22
 <211> 68

<212> DNA
 <213> Homo sapiens

 <400> 22
 cgggatcctt acttgctgct gtcgtccttg tagtccatat gccactgtc taaaggagaa 60
 ttctcaac 68

 <210> 23
 <211> 35
 <212> DNA
 <213> Homo sapiens

 <400> 23
 gcagcagcgg ccgcatgcgc accgtcacca acatc 35

 <210> 24
 <211> 37
 <212> DNA
 <213> Homo sapiens

 <400> 24
 gcagcagtcg acatgcccac tgtctaaagg agaattc 37

 <210> 25
 <211> 39
 <212> DNA
 <213> Homo sapiens

 <400> 25
 gcagcagcgg ccgcatgcag gcgcttaaca ttaccccg 39

 <210> 26
 <211> 34
 <212> DNA
 <213> Homo sapiens

 <400> 26
 gcagcagtcg acatattcct tttcaaaatt actg 34

 <210> 27
 <211> 733
 <212> DNA
 <213> Homo sapiens

 <400> 27
 gggatccgga gcccaaattc tctgacaaaa ctacacatg ccaccgtgc ccagcacctg 60
 aattcgaggg tgcaccgtca gtcttctct tcccccaaa acccaaggac accctcatga 120
 tctcccgac tctgaggtc acatgcgtgg tgggtggacgt aagccacgaa gaccctgagg 180

tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca aagccgcggg	240
aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg caccaggact	300
ggctgaatgg caaggagtac aagtgcaagg tctccaacaa agccctccca acccccatcg	360
agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac accctgcccc	420
catcccggga tgagctgacc aagaaccagg tcagcctgac ctgcctgggc aaaggcttct	480
atccaagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac aactacaaga	540
ccacgcctcc cgtgctggac tccgacggct ccttcttctt ctacagcaag ctcaccgtgg	600
acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat gaggctctgc	660
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gactctagag gat	733

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 <213> Homo sapiens

<400> 28	
caccaaccga agggctttc	19

<210> 29
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<400> 29	
ccacatgggt gatcctacga t	21

<210> 30
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<400> 30	
actgccacca gccagaccac accta	25

<210> 31
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<400> 31	
catccgcctt attacat	17

<210> 32
 <211> 23
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 <213> Homo sapiens

<400> 32
 catccgcctt attacatctt ttt

23

<210> 33
 <211> 17
 <212> DNA
 <213> Homo sapiens

<400> 33
 catgcggggc agcgagg

17

<210> 34
 <211> 24
 <212> DNA
 <213> Homo sapiens

<400> 34
 catgcggggc agcgagggt tcgg

24

<210> 35
 <211> 15
 <212> PRT
 <213> artificial

<220>
 <223> Synthetic Peptide.

<400> 35

Gly Asp Phe Trp Tyr Glu Ala Cys Glu Ser Ser Cys Ala Phe Trp
 1 5 10 15

<210> 36
 <211> 15
 <212> PRT
 <213> artificial

<220>
 <223> Synthetic Peptide.

<400> 36

Leu Glu Trp Gly Ser Asp Val Phe Tyr Asp Val Tyr Asp Cys Cys
 1 5 10 15

<210> 37
 <211> 15
 <212> PRT
 <213> artificial

<220>

<223> Synthetic Peptide.

<400> 37

Cys Leu Arg Ser Gly Thr Gly Cys Ala Phe Gln Leu Tyr Arg Phe
 1 5 10 15

<210> 38

<211> 15

<212> PRT

<213> artificial

<220>

<223> Synthetic Peptide.

<400> 38

Phe Ala Gly Gln Ile Ile Trp Tyr Asp Ala Leu Asp Thr Leu Met
 1 5 10 15

<210> 39

<211> 397

<212> PRT

<213> Homo sapiens

<400> 39

Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu
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Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser
 20 25 30

Ser Lys Gly Arg Ser Leu Ile Gly Lys Val Asp Gly Thr Ser His Val
 35 40 45

Thr Gly Lys Gly Val Thr Val Glu Thr Val Phe Ser Val Asp Glu Phe
 50 55 60

Ser Ala Ser Val Leu Thr Gly Lys Leu Thr Thr Val Phe Leu Pro Ile
 65 70 75 80

Val Tyr Thr Ile Val Phe Val Val Gly Leu Pro Ser Asn Gly Met Ala
 85 90 95

Leu Trp Val Phe Leu Phe Arg Thr Lys Lys Lys His Pro Ala Val Ile
 100 105 110

Tyr Met Ala Asn Leu Ala Leu Ala Asp Leu Leu Ser Val Ile Trp Phe
 115 120 125

Pro Leu Lys Ile Ala Tyr His Ile His Gly Asn Asn Trp Ile Tyr Gly
 130 135 140

Glu Ala Leu Cys Asn Val Leu Ile Gly Phe Phe Tyr Gly Asn Met Tyr

145 150 155 160
 Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val Gln Arg Tyr Trp Val
 165 170 175
 Ile Val Asn Pro Met Gly His Ser Arg Lys Lys Ala Asn Ile Ala Ile
 180 185 190
 Gly Ile Ser Leu Ala Ile Trp Leu Leu Ile Leu Leu Val Thr Ile Pro
 195 200 205
 Leu Tyr Val Val Lys Gln Thr Ile Phe Ile Pro Ala Leu Asn Ile Thr
 210 215 220
 Thr Cys His Asp Val Leu Pro Glu Gln Leu Leu Val Gly Asp Met Phe
 225 230 235 240
 Asn Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe Leu Phe Pro Ala Phe
 245 250 255
 Leu Thr Ala Ser Ala Tyr Val Leu Met Ile Arg Met Leu Arg Ser Ser
 260 265 270
 Ala Met Asp Glu Asn Ser Glu Lys Lys Arg Lys Arg Ala Ile Lys Leu
 275 280 285
 Ile Val Thr Val Leu Ala Met Tyr Leu Ile Cys Phe Thr Pro Ser Asn
 290 295 300
 Leu Leu Leu Val Val His Tyr Phe Leu Ile Lys Ser Gln Gly Gln Ser
 305 310 315 320
 His Val Tyr Ala Leu Tyr Ile Val Ala Leu Cys Leu Ser Thr Leu Asn
 325 330 335
 Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser His Asp Phe Arg
 340 345 350
 Asp His Ala Lys Asn Ala Leu Leu Cys Arg Ser Val Arg Thr Val Lys
 355 360 365
 Gln Met Gln Val Ser Leu Thr Ser Lys Lys His Ser Arg Lys Ser Ser
 370 375 380
 Ser Tyr Ser Ser Ser Ser Thr Thr Val Lys Thr Ser Tyr
 385 390 395

 <210> 40
 <211> 374
 <212> PRT
 <213> Homo sapiens

 <400> 40

 Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Leu Pro
 1 5 10 15

Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys
 20 25 30
 Pro Thr Leu Pro Ile Lys Thr Phe Arg Gly Ala Pro Pro Asn Ser Phe
 35 40 45
 Glu Glu Phe Pro Phe Ser Ala Leu Glu Gly Trp Thr Gly Ala Thr Ile
 50 55 60
 Thr Val Lys Ile Lys Cys Pro Glu Glu Ser Ala Ser His Leu His Val
 65 70 75 80
 Lys Asn Ala Thr Met Gly Tyr Leu Thr Ser Ser Leu Ser Thr Lys Leu
 85 90 95
 Ile Pro Ala Ile Tyr Leu Leu Val Phe Val Val Gly Val Pro Ala Asn
 100 105 110
 Ala Val Thr Leu Trp Met Leu Phe Phe Arg Thr Arg Ser Ile Cys Thr
 115 120 125
 Thr Val Phe Tyr Thr Asn Leu Ala Ile Ala Asp Phe Leu Phe Cys Val
 130 135 140
 Thr Leu Pro Phe Lys Ile Ala Tyr His Leu Asn Gly Asn Asn Trp Val
 145 150 155 160
 Phe Gly Glu Val Leu Cys Arg Ala Thr Thr Val Ile Phe Tyr Gly Asn
 165 170 175
 Met Tyr Cys Ser Ile Leu Leu Leu Ala Cys Ile Ser Ile Asn Arg Tyr
 180 185 190
 Leu Ala Ile Val His Pro Phe Thr Tyr Arg Gly Leu Pro Lys His Thr
 195 200 205
 Tyr Ala Leu Val Thr Cys Gly Leu Val Trp Ala Thr Val Phe Leu Tyr
 210 215 220
 Met Leu Pro Phe Phe Ile Leu Lys Gln Glu Tyr Tyr Leu Val Gln Pro
 225 230 235 240
 Asp Ile Thr Thr Cys His Asp Val His Asn Thr Cys Glu Ser Ser Ser
 245 250 255
 Pro Phe Gln Leu Tyr Tyr Phe Ile Ser Leu Ala Phe Phe Gly Phe Leu
 260 265 270
 Ile Pro Phe Val Leu Ile Ile Tyr Cys Tyr Ala Ala Ile Ile Arg Thr
 275 280 285
 Leu Asn Ala Tyr Asp His Arg Trp Leu Trp Tyr Val Lys Ala Ser Leu
 290 295 300
 Leu Ile Leu Val Ile Phe Thr Ile Cys Phe Ala Pro Ser Asn Ile Ile
 305 310 315 320

Leu Ile Ile His His Ala Asn Tyr Tyr Tyr Asn Asn Thr Asp Gly Leu
 325 330 335

Tyr Phe Ile Tyr Leu Ile Ala Leu Cys Leu Gly Ser Leu Asn Ser Cys
 340 345 350

Leu Asp Pro Phe Leu Tyr Phe Leu Met Ser Lys Thr Arg Asn His Ser
 355 360 365

Thr Ala Tyr Leu Thr Lys
 370

<210> 41
 <211> 420
 <212> PRT
 <213> Xenopus laevis

<400> 41

Met Met Glu Leu Arg Val Leu Leu Leu Leu Leu Leu Thr Leu Leu
 1 5 10 15

Gly Ala Met Gly Ser Leu Cys Leu Ala Asn Ser Asp Thr Gln Ala Lys
 20 25 30

Gly Ala His Ser Asn Asn Met Thr Ile Lys Thr Phe Arg Ile Phe Asp
 35 40 45

Asp Ser Glu Ser Glu Phe Glu Glu Ile Pro Trp Asp Glu Leu Asp Glu
 50 55 60

Ser Gly Glu Gly Ser Gly Asp Gln Ala Pro Val Ser Arg Ser Ala Arg
 65 70 75 80

Lys Pro Ile Arg Arg Asn Ile Thr Lys Glu Ala Glu Gln Tyr Leu Ser
 85 90 95

Ser Gln Trp Leu Thr Lys Phe Val Pro Ser Leu Tyr Thr Val Val Phe
 100 105 110

Ile Val Gly Leu Pro Leu Asn Leu Leu Ala Ile Ile Ile Phe Leu Phe
 115 120 125

Lys Met Lys Val Arg Lys Pro Ala Val Val Tyr Met Leu Asn Leu Ala
 130 135 140

Ile Ala Asp Val Phe Phe Val Ser Val Leu Pro Phe Lys Ile Ala Tyr
 145 150 155 160

His Leu Ser Gly Asn Asp Trp Leu Phe Gly Pro Gly Met Cys Arg Ile
 165 170 175

Val Thr Ala Ile Phe Tyr Cys Asn Met Tyr Cys Ser Val Leu Leu Ile
 180 185 190

Ala Ser Ile Ser Val Asp Arg Phe Leu Ala Val Val Tyr Pro Met His
 195 200 205

Ser Leu Ser Trp Arg Thr Met Ser Arg Ala Tyr Met Ala Cys Ser Phe
 210 215 220
 Ile Trp Leu Ile Ser Ile Ala Ser Thr Ile Pro Leu Leu Val Thr Glu
 225 230 235 240
 Gln Thr Gln Lys Ile Pro Arg Leu Asp Ile Thr Thr Cys His Asp Val
 245 250 255
 Leu Asp Leu Lys Asp Leu Lys Asp Phe Tyr Ile Tyr Tyr Phe Ser Ser
 260 265 270
 Phe Cys Leu Leu Phe Phe Phe Val Pro Phe Ile Ile Thr Thr Ile Cys
 275 280 285
 Tyr Ile Gly Ile Ile Arg Ser Leu Ser Ser Ser Ser Ile Glu Asn Ser
 290 295 300
 Cys Lys Lys Thr Arg Ala Leu Phe Leu Ala Val Val Val Leu Cys Val
 305 310 315 320
 Phe Ile Ile Cys Phe Gly Pro Thr Asn Val Leu Phe Leu Thr His Tyr
 325 330 335
 Leu Gln Glu Ala Asn Glu Phe Leu Tyr Phe Ala Tyr Ile Leu Ser Ala
 340 345 350
 Cys Val Gly Ser Val Ser Cys Cys Leu Asp Pro Leu Ile Tyr Tyr Tyr
 355 360 365
 Ala Ser Ser Gln Cys Gln Arg Tyr Leu Tyr Ser Leu Leu Cys Cys Arg
 370 375 380
 Lys Val Ser Glu Pro Gly Ser Ser Thr Gly Gln Leu Met Ser Thr Ala
 385 390 395 400
 Met Lys Asn Asp Asn Cys Ser Thr Asn Ala Lys Ser Ser Ile Tyr Lys
 405 410 415
 Lys Leu Leu Ala
 420

<210> 42
 <211> 425
 <212> PRT
 <213> Homo sapiens

<400> 42

Met Gly Pro Arg Arg Leu Leu Leu Val Ala Ala Cys Phe Ser Leu Cys
 1 5 10 15
 Gly Pro Leu Leu Ser Ala Arg Thr Arg Ala Arg Arg Pro Glu Ser Lys
 20 25 30
 Ala Thr Asn Ala Thr Leu Asp Pro Arg Ser Phe Leu Leu Arg Asn Pro

35					40					45					
Asn	Asp	Lys	Tyr	Glu	Pro	Phe	Trp	Glu	Asp	Glu	Glu	Lys	Asn	Glu	Ser
50						55					60				
Gly	Leu	Thr	Glu	Tyr	Arg	Leu	Val	Ser	Ile	Asn	Lys	Ser	Ser	Pro	Leu
65					70					75					80
Gln	Lys	Gln	Leu	Pro	Ala	Phe	Ile	Ser	Glu	Asp	Ala	Ser	Gly	Tyr	Leu
				85					90					95	
Thr	Ser	Ser	Trp	Leu	Thr	Leu	Phe	Val	Pro	Ser	Val	Tyr	Thr	Gly	Val
			100					105					110		
Phe	Val	Val	Ser	Leu	Pro	Leu	Asn	Ile	Met	Ala	Ile	Val	Val	Phe	Ile
			115				120					125			
Leu	Lys	Met	Lys	Val	Lys	Lys	Pro	Ala	Val	Val	Tyr	Met	Leu	His	Leu
	130					135					140				
Ala	Thr	Ala	Asp	Val	Leu	Phe	Val	Ser	Val	Leu	Pro	Phe	Lys	Ile	Ser
145					150					155					160
Tyr	Tyr	Phe	Ser	Gly	Ser	Asp	Trp	Gln	Phe	Gly	Ser	Glu	Leu	Cys	Arg
			165						170					175	
Phe	Val	Thr	Ala	Ala	Phe	Tyr	Cys	Asn	Met	Tyr	Ala	Ser	Ile	Leu	Leu
			180					185					190		
Met	Thr	Val	Ile	Ser	Ile	Asp	Arg	Phe	Leu	Ala	Val	Val	Tyr	Pro	Met
	195					200						205			
Gln	Ser	Leu	Ser	Trp	Arg	Thr	Leu	Gly	Arg	Ala	Ser	Phe	Thr	Cys	Leu
	210					215					220				
Ala	Ile	Trp	Ala	Leu	Ala	Ile	Ala	Gly	Val	Val	Pro	Leu	Val	Leu	Lys
225					230					235					240
Glu	Gln	Thr	Ile	Gln	Val	Pro	Gly	Leu	Asn	Ile	Thr	Thr	Cys	His	Asp
				245					250					255	
Val	Leu	Asn	Glu	Thr	Leu	Leu	Glu	Gly	Tyr	Tyr	Ala	Tyr	Tyr	Phe	Ser
			260					265					270		
Ala	Phe	Ser	Ala	Val	Phe	Phe	Phe	Val	Pro	Leu	Ile	Ile	Ser	Thr	Val
			275				280					285			
Cys	Tyr	Val	Ser	Ile	Ile	Arg	Cys	Leu	Ser	Ser	Ser	Ala	Val	Ala	Asn
	290					295					300				
Arg	Ser	Lys	Lys	Ser	Arg	Ala	Leu	Phe	Leu	Ser	Ala	Ala	Val	Phe	Cys
305					310					315					320
Ile	Phe	Ile	Ile	Cys	Phe	Gly	Pro	Thr	Asn	Val	Leu	Leu	Ile	Ala	His
				325					330					335	
Tyr	Ser	Phe	Leu	Ser	His	Thr	Ser	Thr	Thr	Glu	Ala	Ala	Tyr	Phe	Ala

Tyr	Leu	Leu	Cys	Val	Cys	Val	Ser	Ser	Ile	Ser	Ser	Cys	Ile	Asp	Pro	
		355					360					365				
Leu	Ile	Tyr	Tyr	Tyr	Ala	Ser	Ser	Glu	Cys	Gln	Arg	Tyr	Val	Tyr	Ser	
	370					375					380					
Ile	Leu	Cys	Cys	Lys	Glu	Ser	Ser	Asp	Pro	Ser	Ser	Tyr	Asn	Ser	Ser	
385					390					395						400
Gly	Gln	Leu	Met	Ala	Ser	Lys	Met	Asp	Thr	Cys	Ser	Ser	Asn	Leu	Asn	
				405					410					415		
Asn	Ser	Ile	Tyr	Lys	Lys	Leu	Leu	Thr								
			420					425								
<210>	43															
<211>	385															
<212>	PRT															
<213>	Homo sapiens															
<400>	43															
Met	Trp	Gly	Arg	Leu	Leu	Leu	Trp	Pro	Leu	Val	Leu	Gly	Phe	Ser	Leu	
1				5					10					15		
Ser	Gly	Gly	Thr	Gln	Thr	Pro	Ser	Val	Tyr	Asp	Glu	Ser	Gly	Ser	Thr	
			20					25					30			
Gly	Gly	Gly	Asp	Asp	Ser	Thr	Pro	Ser	Ile	Leu	Pro	Ala	Pro	Arg	Gly	
		35					40					45				
Tyr	Pro	Gly	Gln	Val	Cys	Ala	Asn	Asp	Ser	Asp	Thr	Leu	Glu	Leu	Pro	
	50					55					60					
Asp	Ser	Ser	Arg	Ala	Leu	Leu	Leu	Gly	Trp	Val	Pro	Thr	Arg	Leu	Val	
65					70					75					80	
Pro	Ala	Leu	Tyr	Gly	Leu	Val	Leu	Val	Val	Gly	Leu	Pro	Ala	Asn	Gly	
				85					90					95		
Leu	Ala	Leu	Trp	Val	Leu	Ala	Thr	Gln	Ala	Pro	Arg	Leu	Pro	Ser	Thr	
			100					105					110			
Met	Leu	Leu	Met	Asn	Leu	Ala	Thr	Ala	Asp	Leu	Leu	Leu	Ala	Leu	Ala	
	115						120				125					
Leu	Pro	Pro	Arg	Ile	Ala	Tyr	His	Leu	Arg	Gly	Gln	Arg	Trp	Pro	Phe	
	130					135					140					
Gly	Glu	Ala	Ala	Cys	Arg	Leu	Ala	Thr	Ala	Ala	Leu	Tyr	Gly	His	Met	
145					150					155					160	
Tyr	Gly	Ser	Val	Leu	Leu	Leu	Ala	Ala	Val	Ser	Leu	Asp	Arg	Tyr	Leu	
				165					170					175		

Ala Leu Val His Pro Leu Arg Ala Arg Ala Leu Arg Gly Arg Arg Leu
 180 185 190
 Ala Leu Gly Leu Cys Met Ala Ala Trp Leu Met Ala Ala Ala Leu Ala
 195 200 205
 Leu Pro Leu Thr Leu Gln Arg Gln Thr Phe Arg Leu Ala Arg Ser Asp
 210 215 220
 Arg Val Leu Cys His Asp Ala Leu Pro Leu Asp Ala Gln Ala Ser His
 225 230 235 240
 Trp Gln Pro Ala Phe Thr Cys Leu Ala Leu Leu Gly Cys Phe Leu Pro
 245 250 255
 Leu Leu Ala Met Leu Leu Cys Tyr Gly Ala Thr Leu His Thr Leu Ala
 260 265 270
 Ala Ser Gly Arg Arg Tyr Gly His Ala Leu Arg Leu Thr Ala Val Val
 275 280 285
 Leu Ala Ser Ala Val Ala Phe Phe Val Pro Ser Asn Leu Leu Leu Leu
 290 295 300
 Leu His Tyr Ser Asp Pro Ser Pro Ser Ala Trp Gly Asn Leu Tyr Gly
 305 310 315 320
 Ala Tyr Val Pro Ser Leu Ala Leu Ser Thr Leu Asn Ser Cys Val Asp
 325 330 335
 Pro Phe Ile Tyr Tyr Tyr Val Ser Ala Glu Phe Arg Asp Lys Val Arg
 340 345 350
 Ala Gly Leu Phe Gln Arg Ser Pro Gly Asp Thr Val Ala Ser Lys Ala
 355 360 365
 Ser Ala Glu Gly Gly Ser Arg Gly Met Gly Thr His Ser Ser Leu Leu
 370 375 380
 Gln
 385 ..

<210> 44
 <211> 370
 <212> PRT
 <213> Homo sapiens

<400> 44

Met Gly Asp Arg Arg Phe Ile Asp Phe Gln Phe Gln Asp Ser Asn Ser
 1 5 10 15
 Ser Leu Arg Pro Arg Leu Gly Asn Ala Thr Ala Asn Asn Thr Cys Ile
 20 25 30
 Val Asp Asp Ser Phe Lys Tyr Asn Leu Asn Gly Ala Val Tyr Ser Val
 35 40 45

Val Phe Ile Leu Gly Leu Ile Thr Asn Ser Val Ser Leu Phe Val Phe
 50 55 60
 Cys Phe Arg Met Lys Met Arg Ser Glu Thr Ala Ile Phe Ile Thr Asn
 65 70 75 80
 Leu Ala Val Ser Asp Leu Leu Phe Val Cys Thr Leu Pro Phe Lys Ile
 85 90 95
 Phe Tyr Asn Phe Asn Arg His Trp Pro Phe Gly Asp Thr Leu Cys Lys
 100 105 110
 Ile Ser Gly Thr Ala Phe Leu Thr Asn Ile Tyr Gly Ser Met Leu Phe
 115 120 125
 Leu Thr Cys Ile Ser Val Asp Arg Phe Leu Ala Ile Val Tyr Pro Phe
 130 135 140
 Arg Ser Arg Thr Ile Arg Thr Arg Arg Asn Ser Ala Ile Val Cys Ala
 145 150 155 160
 Gly Val Trp Ile Leu Val Leu Ser Gly Gly Ile Ser Ala Ser Leu Phe
 165 170 175
 Ser Thr Thr Asn Val Asn Asn Ala Thr Thr Thr Cys Phe Glu Gly Phe
 180 185 190
 Ser Lys Arg Val Trp Lys Thr Tyr Leu Ser Lys Ile Thr Ile Phe Ile
 195 200 205
 Glu Val Val Gly Phe Ile Ile Pro Leu Ile Leu Asn Val Ser Cys Ser
 210 215 220
 Ser Val Val Leu Arg Thr Leu Arg Lys Pro Ala Thr Leu Ser Gln Ile
 225 230 235 240
 Gly Thr Asn Lys Lys Lys Val Leu Lys Met Ile Thr Val His Met Ala
 245 250 255
 Val Phe Val Val Cys Phe Val Pro Tyr Asn Ser Val Leu Phe Leu Tyr
 260 265 270
 Ala Leu Val Arg Ser Gln Ala Ile Thr Asn Cys Phe Leu Glu Arg Phe
 275 280 285
 Ala Lys Ile Met Tyr Pro Ile Thr Leu Cys Leu Ala Thr Leu Asn Cys
 290 295 300
 Cys Phe Asp Pro Phe Ile Tyr Tyr Phe Thr Leu Glu Ser Phe Gln Lys
 305 310 315 320
 Ser Phe Tyr Ile Asn Ala His Ile Arg Met Glu Ser Leu Phe Lys Thr
 325 330 335
 Glu Thr Pro Leu Thr Thr Lys Pro Ser Leu Pro Ala Ile Gln Glu Glu
 340 345 350

Val Ser Asp Gln Thr Thr Asn Asn Gly Gly Glu Leu Met Leu Glu Ser
 355 360 365

Thr Phe
 370

<210> 45
 <211> 444
 <212> PRT
 <213> Homo sapiens

<400> 45

Met Ser Gly Thr Lys Leu Glu Asp Ser Pro Pro Cys Arg Asn Trp Ser
 1 5 10 15

Ser Ala Ser Glu Leu Asn Glu Thr Gln Glu Pro Phe Leu Asn Pro Thr
 20 25 30

Asp Tyr Asp Asp Glu Glu Phe Leu Arg Tyr Leu Trp Arg Glu Tyr Leu
 35 40 45

His Pro Lys Glu Tyr Glu Trp Val Leu Ile Ala Gly Tyr Ile Ile Val
 50 55 60

Phe Val Val Ala Leu Ile Gly Asn Val Leu Val Cys Val Ala Val Trp
 65 70 75 80

Lys Asn His His Met Arg Thr Val Thr Asn Tyr Phe Ile Val Asn Leu
 85 90 95

Ser Leu Ala Asp Val Leu Val Thr Ile Thr Cys Leu Pro Ala Thr Leu
 100 105 110

Val Val Asp Ile Thr Glu Thr Trp Phe Phe Gly Gln Ser Leu Cys Lys
 115 120 125

Val Ile Pro Tyr Leu Gln Thr Val Ser Val Ser Val Ser Val Leu Thr
 130 135 140

Leu Ser Cys Ile Ala Leu Asp Arg Trp Tyr Ala Ile Cys His Pro Leu
 145 150 155 160

Met Phe Lys Ser Thr Ala Lys Arg Ala Arg Asn Ser Ile Val Ile Ile
 165 170 175

Trp Ile Val Ser Cys Ile Ile Met Ile Pro Gln Ala Ile Val Met Glu
 180 185 190

Cys Ser Thr Val Phe Pro Gly Leu Ala Asn Lys Thr Thr Leu Phe Thr
 195 200 205

Val Cys Asp Glu Arg Trp Gly Gly Glu Ile Tyr Pro Lys Met Tyr His
 210 215 220

Ile Cys Phe Phe Leu Val Thr Tyr Met Ala Pro Leu Cys Leu Met Val

225 230 235 240
 Leu Ala Tyr Leu Gln Ile Phe Arg Lys Leu Trp Cys Arg Gln Ile Pro
 245 250 255
 Gly Thr Ser Ser Val Val Gln Arg Lys Trp Lys Pro Leu Gln Pro Val
 260 265 270
 Ser Gln Pro Arg Gly Pro Gly Gln Pro Thr Lys Ser Arg Met Ser Ala
 275 280 285
 Val Ala Ala Glu Ile Lys Gln Ile Arg Ala Arg Arg Lys Thr Ala Arg
 290 295 300
 Met Leu Met Val Val Leu Leu Val Phe Ala Ile Cys Tyr Leu Pro Ile
 305 310 315 320
 Ser Ile Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Ala His Thr
 325 330 335
 Glu Asp Arg Glu Thr Val Tyr Ala Trp Phe Thr Phe Ser His Trp Leu
 340 345 350
 Val Tyr Ala Asn Ser Ala Ala Asn Pro Ile Ile Tyr Asn Phe Leu Ser
 355 360 365
 Gly Lys Phe Arg Glu Glu Phe Lys Ala Ala Phe Ser Cys Cys Cys Leu
 370 375 380
 Gly Val His His Arg Gln Glu Asp Arg Leu Thr Arg Gly Arg Thr Ser
 385 390 395 400
 Thr Glu Ser Arg Lys Ser Leu Thr Thr Gln Ile Ser Asn Phe Asp Asn
 405 410 415
 Ile Ser Lys Leu Ser Glu Gln Val Val Leu Thr Ser Ile Ser Thr Leu
 420 425 430
 Pro Ala Ala Asn Gly Ala Gly Pro Leu Gln Asn Trp
 435 440

<210> 46
 <211> 460
 <212> PRT
 <213> Rattus norvegicus

<400> 46

Met Ser Ser Thr Lys Leu Glu Asp Ser Leu Pro Arg Arg Asn Trp Ser
 1 5 10 15
 Ser Ala Ser Glu Leu Asn Glu Thr Gln Glu Pro Phe Leu Asn Pro Thr
 20 25 30
 Asp Tyr Asp Asp Glu Glu Phe Leu Arg Tyr Leu Trp Arg Glu Tyr Leu
 35 40 45

His Pro Lys Glu Tyr Glu Trp Val Leu Ile Ala Gly Tyr Ile Ile Val
 50 55 60
 Phe Val Val Ala Leu Ile Gly Asn Val Leu Val Cys Val Ala Val Trp
 65 70 75 80
 Lys Asn His His Met Arg Thr Val Thr Asn Tyr Phe Ile Val Asn Leu
 85 90 95
 Ser Leu Ala Asp Val Leu Val Thr Ile Thr Cys Leu Pro Ala Thr Leu
 100 105 110
 Val Val Asp Ile Thr Glu Thr Trp Phe Phe Gly Gln Ser Leu Cys Lys
 115 120 125
 Val Ile Pro Tyr Leu Gln Thr Val Ser Val Ser Val Ser Val Leu Thr
 130 135 140
 Leu Ser Cys Ile Ala Leu Asp Arg Trp Tyr Ala Ile Cys His Pro Leu
 145 150 155 160
 Met Phe Lys Ser Thr Ala Lys Arg Ala Arg Asn Ser Ile Val Val Ile
 165 170 175
 Trp Ile Val Ser Cys Ile Ile Met Ile Pro Gln Ala Ile Val Met Glu
 180 185 190
 Arg Ser Ser Met Leu Pro Gly Leu Ala Asn Lys Thr Thr Leu Phe Thr
 195 200 205
 Val Cys Asp Glu Arg Trp Gly Gly Glu Val Tyr Pro Lys Met Tyr His
 210 215 220
 Ile Cys Phe Phe Leu Val Thr Tyr Met Ala Pro Leu Cys Leu Met Val
 225 230 235 240
 Leu Ala Tyr Leu Gln Ile Phe Arg Lys Leu Trp Cys Arg Gln Ile Pro
 245 250 255
 Gly Thr Ser Ser Val Val Gln Arg Lys Trp Lys Gln Pro Gln Pro Val
 260 265 270
 Ser Gln Pro Arg Gly Ser Gly Gln Gln Ser Lys Ala Arg Ile Ser Ala
 275 280 285
 Val Ala Ala Glu Ile Lys Gln Ile Arg Ala Arg Arg Lys Thr Ala Arg
 290 295 300
 Met Leu Met Val Val Leu Leu Val Phe Ala Ile Cys Tyr Leu Pro Ile
 305 310 315 320
 Ser Ile Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Thr His Thr
 325 330 335
 Glu Asp Arg Glu Thr Val Tyr Ala Trp Phe Thr Phe Ser His Trp Leu
 340 345 350

Val Tyr Ala Asn Ser Ala Ala Asn Pro Ile Ile Tyr Asn Phe Leu Ser
 355 360 365

Gly Lys Phe Arg Glu Glu Phe Lys Ala Ala Phe Ser Cys Cys Leu Gly
 370 375 380

Val His Arg Arg Gln Gly Asp Arg Leu Ala Arg Gly Arg Thr Ser Thr
 385 390 395 400

Glu Ser Arg Lys Ser Leu Thr Thr Gln Ile Ser Asn Phe Asp Asn Val
 405 410 415

Ser Lys Leu Ser Glu His Val Ala Leu Thr Ser Ile Ser Thr Leu Pro
 420 425 430

Ala Ala Asn Gly Ala Gly Pro Leu Gln Asn Trp Tyr Leu Gln Gln Gly
 435 440 445

Val Pro Ser Ser Leu Leu Ser Thr Trp Leu Glu Val
 450 455 460

<210> 47
 <211> 375
 <212> PRT
 <213> Mus musculus

<400> 47

Met Asn Thr Ser His Phe Leu Ala Pro Leu Phe Pro Gly Ser Leu Gln
 1 5 10 15

Gly Lys Asn Gly Thr Asn Pro Leu Asp Ser Pro Tyr Asn Phe Ser Asp
 20 25 30

Gly Cys Gln Asp Ser Ala Glu Leu Leu Ala Phe Ile Ile Thr Thr Tyr
 35 40 45

Ser Ile Glu Thr Ile Leu Gly Val Leu Gly Asn Leu Cys Leu Ile Phe
 50 55 60

Val Thr Thr Arg Gln Lys Glu Lys Ser Asn Val Thr Asn Leu Leu Ile
 65 70 75 80

Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Ile Cys Gln Pro
 85 90 95

Leu Thr Val Thr Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Val
 100 105 110

Leu Cys Lys Met Leu Thr Phe Ile Gln Cys Met Ser Val Thr Val Ser
 115 120 125

Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile
 130 135 140

Asn Pro Thr Gly Trp Lys Pro Ser Ile Phe Gln Ala Tyr Leu Gly Ile
 145 150 155 160

Val Val Ile Trp Phe Ile Ser Cys Phe Leu Ser Leu Pro Phe Leu Ala
 165 170 175
 Asn Ser Thr Leu Asn Asp Leu Phe His Tyr Asn His Ser Lys Val Val
 180 185 190
 Glu Phe Leu Glu Asp Lys Val Val Cys Phe Val Ser Trp Ser Ser Asp
 195 200 205
 His His Arg Leu Ile Tyr Thr Thr Phe Leu Leu Leu Phe Gln Tyr Cys
 210 215 220
 Ile Pro Leu Ala Phe Ile Leu Val Cys Tyr Ile Arg Ile Tyr Gln Arg
 225 230 235 240
 Leu Gln Arg Gln Lys His Val Phe His Ala His Ala Cys Ser Ser Arg
 245 250 255
 Ala Gly Gln Met Lys Arg Ile Asn Ser Met Leu Met Thr Met Val Thr
 260 265 270
 Ala Phe Ala Val Leu Trp Leu Pro Leu His Val Phe Asn Thr Leu Glu
 275 280 285
 Asp Trp Tyr Gln Glu Ala Ile Pro Ala Cys His Gly Asn Leu Ile Phe
 290 295 300
 Leu Met Cys His Leu Leu Ala Met Ala Ser Thr Cys Val Asn Pro Phe
 305 310 315 320
 Ile Tyr Gly Phe Leu Asn Ile Asn Phe Lys Lys Asp Ile Lys Ala Leu
 325 330 335
 Val Leu Thr Cys His Cys Arg Ser Pro Gln Gly Glu Ser Glu His Leu
 340 345 350
 Pro Leu Ser Thr Val His Thr Asp Leu Ser Lys Gly Ser Met Arg Met
 355 360 365
 Gly Ser Lys Ser Asn Phe Ile
 370 375
 <210> 48
 <211> 375
 <212> PRT
 <213> Rattus norvegicus
 <400> 48
 Met Asn Thr Ser His Leu Met Ala Ser Leu Ser Pro Ala Phe Leu Gln
 1 5 10 15
 Gly Lys Asn Gly Thr Asn Pro Leu Asp Ser Leu Tyr Asn Leu Ser Asp
 20 25 30
 Gly Cys Gln Asp Ser Ala Asp Leu Leu Ala Phe Ile Ile Thr Thr Tyr

35					40					45					
Ser	Val	Glu	Thr	Val	Leu	Gly	Val	Leu	Gly	Asn	Leu	Cys	Leu	Ile	Phe
50						55					60				
Val	Thr	Thr	Arg	Gln	Lys	Glu	Lys	Ser	Asn	Val	Thr	Asn	Leu	Leu	Ile
65					70					75					80
Ala	Asn	Leu	Ala	Phe	Ser	Asp	Phe	Leu	Met	Cys	Leu	Ile	Cys	Gln	Pro
				85					90					95	
Leu	Thr	Val	Thr	Tyr	Thr	Ile	Met	Asp	Tyr	Trp	Ile	Phe	Gly	Glu	Val
			100					105					110		
Leu	Cys	Lys	Met	Leu	Thr	Phe	Ile	Gln	Cys	Met	Ser	Val	Thr	Val	Ser
		115					120					125			
Ile	Leu	Ser	Leu	Val	Leu	Val	Ala	Leu	Glu	Arg	His	Gln	Leu	Ile	Ile
	130					135					140				
Asn	Pro	Thr	Gly	Trp	Lys	Pro	Ser	Ile	Ser	Gln	Ala	Tyr	Leu	Gly	Ile
145					150					155					160
Val	Val	Ile	Trp	Phe	Ile	Ser	Cys	Phe	Leu	Ser	Leu	Pro	Phe	Leu	Ala
				165					170					175	
Asn	Ser	Ile	Leu	Asn	Asp	Leu	Phe	His	Tyr	Asn	His	Ser	Lys	Val	Val
			180					185					190		
Glu	Phe	Leu	Glu	Asp	Lys	Val	Val	Cys	Phe	Val	Ser	Trp	Ser	Ser	Asp
		195					200					205			
His	His	Arg	Leu	Ile	Tyr	Thr	Thr	Phe	Leu	Leu	Leu	Phe	Gln	Tyr	Cys
	210					215					220				
Val	Pro	Leu	Ala	Phe	Ile	Leu	Val	Cys	Tyr	Met	Arg	Ile	Tyr	Gln	Arg
225					230					235					240
Leu	Gln	Arg	Gln	Arg	Arg	Ala	Phe	His	Thr	His	Thr	Cys	Ser	Ser	Arg
				245					250					255	
Val	Gly	Gln	Met	Lys	Arg	Ile	Asn	Gly	Met	Leu	Met	Ala	Met	Val	Thr
			260					265					270		
Ala	Phe	Ala	Val	Leu	Trp	Leu	Pro	Leu	His	Val	Phe	Asn	Thr	Leu	Glu
		275					280					285			
Asp	Trp	Tyr	Gln	Glu	Ala	Ile	Pro	Ala	Cys	His	Gly	Asn	Leu	Ile	Phe
	290					295					300				
Leu	Met	Cys	His	Leu	Phe	Ala	Met	Ala	Ser	Thr	Cys	Val	Asn	Pro	Phe
305					310					315					320
Ile	Tyr	Gly	Phe	Leu	Asn	Ile	Asn	Phe	Lys	Lys	Asp	Ile	Lys	Ala	Leu
				325					330					335	
Val	Leu	Thr	Cys	Arg	Cys	Arg	Pro	Pro	Gln	Gly	Glu	Pro	Glu	Pro	Leu

340	345	350
Pro Leu Ser Thr Val His Thr Asp Leu Ser Lys Gly Ser Met Arg Met		
355	360	365
Gly Ser Lys Ser Asn Val Met		
370	375	
<210> 49		
<211> 371		
<212> PRT		
<213> Oryctolagus cuniculus		
<400> 49		
Met Glu Val Ser Leu Asn Asp Pro Ala Ser Asn Lys Thr Ser Ala Lys		
1	5	10
Ser Asn Ser Ser Ala Phe Phe Tyr Phe Glu Ser Cys Gln Ser Pro Ser		
20	25	30
Leu Ala Leu Leu Leu Leu Leu Ile Ala Tyr Thr Val Val Leu Ile Met		
35	40	45
Gly Ile Cys Gly Asn Leu Ser Leu Ile Thr Ile Ile Phe Lys Lys Gln		
50	55	60
Arg Glu Ala Gln Asn Val Thr Asn Ile Leu Ile Ala Asn Leu Ser Leu		
65	70	75
Ser Asp Ile Leu Val Cys Val Met Cys Ile Pro Phe Thr Ala Ile Tyr		
85	90	95
Thr Leu Met Asp Arg Trp Ile Phe Gly Asn Thr Met Cys Lys Leu Thr		
100	105	110
Ser Tyr Val Gln Ser Val Ser Ile Ser Val Ser Ile Phe Ser Leu Val		
115	120	125
Leu Ile Ala Ile Glu Arg Tyr Gln Leu Ile Val Asn Pro Arg Gly Trp		
130	135	140
Lys Pro Ser Ala Ser His Ala Tyr Trp Gly Ile Met Leu Ile Trp Leu		
145	150	155
Phe Ser Leu Leu Leu Ser Ile Pro Leu Leu Leu Ser Tyr His Leu Thr		
165	170	175
Asp Glu Pro Phe Arg Asn Leu Ser Leu Pro Thr Asp Leu Tyr Ser His		
180	185	190
His Val Val Cys Val Glu His Trp Pro Ser Lys Thr Asn Gln Leu Leu		
195	200	205
Tyr Ser Thr Ser Leu Ile Met Leu Gln Tyr Phe Val Pro Leu Gly Phe		
210	215	220

Met Phe Ile Cys Tyr Leu Lys Ile Val Ile Cys Leu His Lys Arg Asn
 225 230 235 240
 Ser Lys Ile Asp Arg Arg Arg Glu Asn Glu Ser Arg Leu Thr Glu Asn
 245 250 255
 Lys Arg Ile Asn Thr Met Leu Ile Ser Ile Val Val Thr Phe Ala Ala
 260 265 270
 Cys Trp Leu Pro Leu Asn Thr Phe Asn Val Ile Phe Asp Trp Tyr His
 275 280 285
 Glu Val Leu Met Ser Cys His His Asp Leu Val Phe Ala Ile Cys His
 290 295 300
 Leu Val Ala Met Val Ser Thr Cys Ile Asn Pro Leu Phe Tyr Gly Phe
 305 310 315 320
 Leu Asn Arg Asn Phe Gln Lys Asp Leu Val Val Leu Ile His His Cys
 325 330 335
 Leu Cys Phe Ala Leu Arg Glu Arg Tyr Glu Asn Ile Ala Ile Ser Thr
 340 345 350
 Leu His Thr Asp Glu Ser Lys Gly Ser Leu Arg Val Ala His Ile Pro
 355 360 365
 Ala Gly Ile
 370

<210> 50
 <211> 383
 <212> PRT
 <213> Cavia porcellus

<400> 50

Met Asn Ser Thr Ser Phe Ser Gln Leu Glu Asn His Ser Val His Tyr
 1 5 10 15
 Asn Leu Ser Glu Glu Lys Pro Ser Phe Phe Ala Phe Glu Asn Asp Asp
 20 25 30
 Cys His Leu Pro Leu Ala Val Ile Phe Thr Leu Ala Leu Ala Tyr Gly
 35 40 45
 Ala Val Ile Ile Leu Gly Val Ser Gly Asn Leu Ala Leu Ile Leu Ile
 50 55 60
 Ile Leu Lys Gln Lys Glu Met Arg Asn Val Thr Asn Ile Leu Ile Val
 65 70 75 80
 Asn Leu Ser Phe Ser Asp Leu Leu Val Ala Ile Met Cys Leu Pro Phe
 85 90 95
 Thr Phe Val Tyr Thr Leu Met Asp His Trp Ile Phe Gly Glu Ile Met
 100 105 110

Cys Lys Leu Asn Pro Phe Val Gln Cys Val Ser Ile Thr Val Ser Ile
 115 120 125
 Phe Ser Leu Val Leu Ile Ala Val Glu Arg His Gln Leu Ile Ile Asn
 130 135 140
 Pro Arg Gly Trp Arg Pro Asn Asn Arg His Ala Tyr Ile Gly Ile Ala
 145 150 155 160
 Val Ile Trp Val Leu Ala Val Ala Ser Ser Leu Pro Phe Met Ile Tyr
 165 170 175
 Gln Val Leu Thr Asp Glu Pro Phe Gln Asn Val Thr Leu Asp Ala Phe
 180 185 190
 Lys Asp Lys Leu Val Cys Phe Asp Gln Phe Pro Ser Asp Ser His Arg
 195 200 205
 Leu Ser Tyr Thr Thr Leu Leu Leu Val Leu Gln Tyr Phe Gly Pro Leu
 210 215 220
 Cys Phe Ile Phe Ile Cys Tyr Phe Lys Ile Tyr Ile Arg Leu Lys Arg
 225 230 235 240
 Arg Asn Asn Met Met Asp Lys Met Arg Asp Ser Lys Tyr Arg Ser Ser
 245 250 255
 Glu Ser Lys Arg Ile Asn Ile Met Leu Leu Ser Ile Val Val Ala Phe
 260 265 270
 Ala Val Cys Trp Leu Pro Leu Thr Ile Phe Asn Thr Val Phe Asp Trp
 275 280 285
 Asn His Gln Ile Ile Ala Thr Cys Asn His Asn Leu Leu Phe Leu Leu
 290 295 300
 Cys His Leu Thr Ala Met Ile Ser Thr Cys Val Asn Pro Ile Phe Tyr
 305 310 315 320
 Gly Phe Leu Asn Lys Asn Phe Gln Arg Asp Leu Gln Phe Phe Phe Asn
 325 330 335
 Phe Cys Asp Phe Arg Ser Arg Asp Asp Asp Tyr Glu Thr Ile Ala Met
 340 345 350
 Ser Thr Met His Thr Asp Val Ser Lys Thr Ser Leu Lys Gln Ala Ser
 355 360 365
 Pro Leu Ala Phe Lys Lys Ile Ser Cys Val Glu Asn Glu Lys Ile
 370 375 380

<210> 51
 <211> 375
 <212> PRT
 <213> Danio rerio

<400> 51

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Met Glu Arg Ser His Leu Asn Asn Ser Ser Trp Leu Leu Glu Asp Pro
1      5      10      15
Thr Cys Pro Ala Ser Leu Ser Ser Thr Thr Phe Leu Ile Val Ala Tyr
20      25      30
Ser Thr Met Leu Ala Val Gly Leu Val Gly Asn Thr Cys Leu Val Val
35      40      45
Val Ile Thr Arg Gln Lys Glu Met Arg Asn Val Thr Asn Ile Phe Ile
50      55      60
Val Asn Leu Ser Cys Ser Asp Ile Leu Val Cys Leu Val Cys Leu Pro
65      70      75      80
Val Thr Ile Ile Tyr Thr Leu Met Asp Arg Trp Ile Leu Gly Glu Ala
85      90      95
Leu Cys Lys Val Thr Pro Phe Val Gln Cys Met Ser Val Thr Val Ser
100     105     110
Ile Phe Ser Met Val Leu Ile Ala Leu Glu Arg His Gln Leu Ile Ile
115     120     125
His Pro Thr Gly Trp Lys Pro Val Val Arg His Ser Tyr Leu Ala Val
130     135     140
Ala Val Ile Trp Ile Ile Ala Cys Phe Ile Ser Leu Pro Phe Leu Ser
145     150     155     160
Phe Asn Ile Leu Thr Asn Ser Pro Phe His Asn Leu Ser Leu Pro Phe
165     170     175
Asn Pro Phe Ser Asp His Phe Ile Cys Ile Glu Gln Trp Pro Ser Glu
180     185     190
Gly Asn Arg Leu Thr Tyr Thr Thr Thr Leu Leu Leu Cys Gln Tyr Cys
195     200     205
Leu Pro Leu Ala Leu Ile Leu Val Cys Tyr Phe Arg Ile Phe Leu Arg
210     215     220
Leu Ser Arg Arg Lys Asp Met Val Glu Arg Ala Arg Gly Gly Arg Gln
225     230     235     240
Lys Lys Ala Lys Gly Ser Lys Arg Val Asn Ala Met Leu Ala Ser Ile
245     250     255
Val Ala Ala Phe Ala Leu Cys Trp Leu Pro Leu Asn Val Phe Asn Thr
260     265     270
Ile Phe Asp Trp Asn His Glu Ala Ile Pro Val Cys Gln His Asp Ala
275     280     285
Ile Phe Ser Ala Cys His Leu Thr Ala Met Ala Ser Thr Cys Val Asn

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290 295 300
 Pro Val Ile Tyr Gly Phe Leu Asn Asn Asn Phe Gln Lys Glu Leu Lys
 305 310 315 320
 Ser Leu Leu Ser Arg Cys Arg Cys Trp Gly Pro Ala Glu Ser Tyr Glu
 325 330 335
 Ser Phe Pro Leu Ser Thr Val Ser Thr Gly Ile Thr Lys Gly Ser Ile
 340 345 350
 Leu Ser Asn Gly Ser Ala Ser Thr Tyr Gln Pro His Lys Lys Asn Ser
 355 360 365
 Leu Glu Gln Lys Glu Ser Ile
 370 375
 <210> 52
 <211> 381
 <212> PRT
 <213> Homo sapiens
 <400> 52
 Met Gly Pro Ile Gly Ala Glu Ala Asp Glu Asn Gln Thr Val Glu Glu
 1 5 10 15
 Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu Leu
 20 25 30
 Val Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile Glu
 35 40 45
 Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly
 50 55 60
 Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser
 65 70 75 80
 Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp
 85 90 95
 Leu Leu Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu
 100 105 110
 Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr
 115 120 125
 Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile
 130 135 140
 Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile
 145 150 155 160
 Ser Lys Arg Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile Ser
 165 170 175

Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile
 180 185 190
 Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro
 195 200 205
 Gly Glu Glu Lys Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser Leu
 210 215 220
 Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr
 225 230 235 240
 Arg Ile Trp Ser Lys Leu Lys Asn His Val Ser Pro Gly Ala Ala Asn
 245 250 255
 Asp His Tyr His Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val Cys
 260 265 270
 Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln
 275 280 285
 Leu Ala Val Asp Ile Asp Ser Gln Val Leu Asp Leu Lys Glu Tyr Lys
 290 295 300
 Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala
 305 310 315 320
 Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe
 325 330 335
 Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu
 340 345 350
 Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys Asn
 355 360 365
 Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val
 370 375 380
 <210> 53
 <211> 522
 <212> PRT
 <213> Homo sapiens
 <400> 53

Met Asn Ser Phe Phe Gly Thr Pro Ala Ala Ser Trp Cys Leu Leu Glu
 1 5 10 15
 Ser Asp Val Ser Ser Ala Pro Asp Lys Glu Ala Gly Arg Glu Arg Arg
 20 25 30
 Ala Leu Ser Val Gln Gln Arg Gly Gly Pro Ala Trp Ser Gly Ser Leu
 35 40 45
 Glu Trp Ser Arg Gln Ser Ala Gly Asp Arg Arg Arg Leu Gly Leu Ser
 50 55 60

Arg Gln Thr Ala Lys Ser Ser Trp Ser Arg Ser Arg Asp Arg Thr Cys
 65 70 75 80
 Cys Cys Arg Arg Ala Trp Trp Ile Leu Val Pro Ala Ala Asp Arg Ala
 85 90 95
 Arg Arg Glu Arg Phe Ile Met Asn Glu Lys Trp Asp Thr Asn Ser Ser
 100 105 110
 Glu Asn Trp His Pro Ile Trp Asn Val Asn Asp Thr Lys His His Leu
 115 120 125
 Tyr Ser Asp Ile Asn Ile Thr Tyr Val Asn Tyr Tyr Leu His Gln Pro
 130 135 140
 Gln Val Ala Ala Ile Phe Ile Ile Ser Tyr Phe Leu Ile Phe Phe Leu
 145 150 155 160
 Cys Met Met Gly Asn Thr Val Val Cys Phe Ile Val Met Arg Asn Lys
 165 170 175
 His Met His Thr Val Thr Asn Leu Phe Ile Leu Asn Leu Ala Ile Ser
 180 185 190
 Asp Leu Leu Val Gly Ile Phe Cys Met Pro Ile Thr Leu Leu Asp Asn
 195 200 205
 Ile Ile Ala Gly Trp Pro Phe Gly Asn Thr Met Cys Lys Ile Ser Gly
 210 215 220
 Leu Val Gln Gly Ile Ser Val Ala Ala Ser Val Phe Thr Leu Val Ala
 225 230 235 240
 Ile Ala Val Asp Arg Phe Gln Cys Val Val Tyr Pro Phe Lys Pro Lys
 245 250 255
 Leu Thr Ile Lys Thr Ala Phe Val Ile Ile Met Ile Ile Trp Val Leu
 260 265 270
 Ala Ile Thr Ile Met Ser Pro Ser Ala Val Met Leu His Val Gln Glu
 275 280 285
 Glu Lys Tyr Tyr Arg Val Arg Leu Asn Ser Gln Asn Lys Thr Ser Pro
 290 295 300
 Val Tyr Trp Cys Arg Glu Asp Trp Pro Asn Gln Glu Met Arg Lys Ile
 305 310 315 320
 Tyr Thr Thr Val Leu Phe Ala Asn Ile Tyr Leu Ala Pro Leu Ser Leu
 325 330 335
 Ile Val Ile Met Tyr Gly Arg Ile Gly Ile Ser Leu Phe Arg Ala Ala
 340 345 350
 Val Pro His Thr Gly Arg Lys Asn Gln Glu Gln Trp His Val Val Ser
 355 360 365

Arg Lys Lys Gln Lys Ile Ile Lys Met Leu Leu Ile Val Ala Leu Leu
 370 375 380
 Phe Ile Leu Ser Trp Leu Pro Leu Trp Thr Leu Met Met Leu Ser Asp
 385 390 395 400
 Tyr Ala Asp Leu Ser Pro Asn Glu Leu Gln Ile Ile Asn Ile Tyr Ile
 405 410 415
 Tyr Pro Phe Ala His Trp Leu Ala Phe Gly Asn Ser Ser Val Asn Pro
 420 425 430
 Ile Ile Tyr Gly Phe Phe Asn Glu Asn Phe Arg Arg Gly Phe Gln Glu
 435 440 445
 Ala Phe Gln Leu Gln Leu Cys Gln Lys Arg Ala Lys Pro Met Glu Ala
 450 455 460
 Tyr Thr Leu Lys Ala Lys Ser His Val Leu Ile Asn Thr Ser Asn Gln
 465 470 475 480
 Leu Val Gln Glu Ser Thr Phe Gln Asn Pro His Gly Glu Thr Leu Leu
 485 490 495
 Tyr Arg Lys Ser Ala Glu Lys Pro Gln Gln Glu Leu Val Met Glu Glu
 500 505 510
 Leu Lys Glu Thr Thr Asn Ser Ser Glu Ile
 515 520

<210> 54
 <211> 348
 <212> PRT
 <213> Mus musculus

<400> 54

Met Glu Leu Ala Met Val Asn Leu Ser Glu Gly Asn Gly Ser Asp Pro
 1 5 10 15
 Glu Pro Pro Ala Pro Glu Ser Arg Pro Leu Phe Gly Ile Gly Val Glu
 20 25 30
 Asn Phe Ile Thr Leu Val Val Phe Gly Leu Ile Phe Ala Met Gly Val
 35 40 45
 Leu Gly Asn Ser Leu Val Ile Thr Val Leu Ala Arg Ser Lys Pro Gly
 50 55 60
 Lys Pro Arg Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Ser Ile Ala
 65 70 75 80
 Asp Leu Ala Tyr Leu Leu Phe Cys Ile Pro Phe Gln Ala Thr Val Tyr
 85 90 95
 Ala Leu Pro Thr Trp Val Leu Gly Ala Phe Ile Cys Lys Phe Ile His

100					105					110					
Tyr	Phe	Phe	Thr	Val	Ser	Met	Leu	Val	Ser	Ile	Phe	Thr	Leu	Ala	Ala
	115						120					125			
Met	Ser	Val	Asp	Arg	Tyr	Val	Ala	Ile	Val	His	Ser	Arg	Arg	Ser	Ser
	130					135					140				
Ser	Leu	Arg	Val	Ser	Arg	Asn	Ala	Leu	Leu	Gly	Val	Gly	Phe	Ile	Trp
	145					150					155				160
Ala	Leu	Ser	Ile	Ala	Met	Ala	Ser	Pro	Val	Ala	Tyr	His	Gln	Arg	Leu
				165					170					175	
Phe	His	Arg	Asp	Ser	Asn	Gln	Thr	Phe	Cys	Trp	Glu	Gln	Trp	Pro	Asn
			180					185					190		
Lys	Leu	His	Lys	Lys	Ala	Tyr	Val	Val	Cys	Thr	Phe	Val	Phe	Gly	Tyr
		195					200					205			
Leu	Leu	Pro	Leu	Leu	Leu	Ile	Cys	Phe	Cys	Tyr	Ala	Lys	Val	Leu	Asn
	210					215					220				
His	Leu	His	Lys	Lys	Leu	Lys	Asn	Met	Ser	Lys	Lys	Ser	Glu	Ala	Ser
	225					230					235				240
Lys	Lys	Lys	Thr	Ala	Gln	Thr	Val	Leu	Val	Val	Val	Val	Val	Phe	Gly
				245					250					255	
Ile	Ser	Trp	Leu	Pro	His	His	Val	Val	His	Leu	Trp	Ala	Glu	Phe	Gly
			260					265					270		
Ala	Phe	Pro	Leu	Thr	Pro	Ala	Ser	Phe	Phe	Phe	Arg	Ile	Thr	Ala	His
		275					280					285			
Cys	Leu	Ala	Tyr	Ser	Asn	Ser	Ser	Val	Asn	Pro	Ile	Ile	Tyr	Ala	Phe
	290					295					300				
Leu	Ser	Glu	Asn	Phe	Arg	Lys	Ala	Tyr	Lys	Gln	Val	Phe	Lys	Cys	His
	305					310					315				320
Val	Cys	Asp	Glu	Ser	Pro	Arg	Ser	Glu	Thr	Lys	Glu	Asn	Lys	Ser	Arg
				325					330					335	
Met	Asp	Thr	Pro	Pro	Ser	Thr	Asn	Cys	Thr	His	Val				
			340					345							

<210> 55

<211> 38

<212> DNA

<213> Homo sapiens

<400> 55

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38

<210> 56

<211> 37
<212> DNA
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<400> 56
gcagcagtcg acgaacacac tctcctgcct ctggagg 37

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<211> 39
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<400> 57
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<210> 58
<211> 37
<212> DNA
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<400> 58
gcagcagtcg accttgtaga cgtggtagta gctcttg 37